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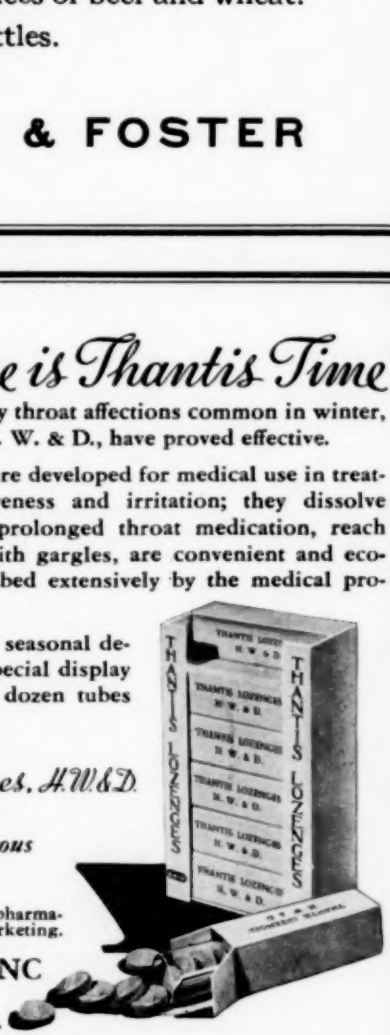

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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The Bioassay of Aconite*,†

*By B. V. Christensen‡ and J. W. Nelson***

INTRODUCTION

Although *Aconitum napellus* has been official in every U. S. Pharmacopœia from 1820 to 1930, inclusive, there has never yet been devised a completely satisfactory method for its standardization. Aconitine is widely recognized as one of the most potent alkaloids known; consequently, any assay procedure which gives a product of variable toxicity is dangerous. It has been largely because of this fact that aconite has recently fallen into disuse.

It has been fairly well established that the chief active alkaloids of aconite are aconitine, benzoylaconine and aconine. Pharmacologic tests indicate that aconitine is about 500 times as toxic as benzoylaconine, and 5000 times as toxic as aconine. And it is quite generally conceded that the activity of aconite is due to aconitine, which is the chief alkaloid both qualitatively and quantitatively.

Aconite has been suggested for many and varied therapeutic uses; however, since its chief therapeutic uses depend upon its analgesic action, it seemed worth while to conduct a preliminary investigation to ascertain whether this analgesic action is due to aconitine or some other plant constituent or constituents. Having satisfactorily determined the analgesic agent in aconite a suitable assay procedure was then sought.

A great amount of work has been done on the standardization of aconite and, since none of the methods devised have proved entirely satisfactory, it was deemed worth while to attempt a different method hitherto untried rather than to try to improve on some one of the more or less unsuccessful methods.

It has already been shown (1) that aconite will produce emesis and since the pigeon emesis method of assay has recently been applied to several drugs with success, it appeared logical that aconite might be standardized by this method. Therefore the second part of this work was undertaken to determine if the pigeon emesis method was applicable to the assay of aconite.

It is recognized that a chemical method of assay is most desirable when such an assay is

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* Scientific Section, A. PH. A., Atlanta meeting, 1939.

† Abstract of a thesis presented to the Graduate Council of the University of Florida by J. W. Nelson in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy.

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possible; however, aconite appears to be one of those drugs that does not lend itself to a chemical assay in that to date no satisfactory method of separating the active constituents has been devised. It has already been pointed out that an assay based on total alkaloidal content is useless. It therefore appears that a biological assay must be resorted to.

It is also recognized that, for a biological assay to be justified, it must be practical in that it must possess the following virtues: simplicity, rapidity, economy, a definite and unmistakable end-point and a reasonable degree of accuracy.

The chief physiological tests heretofore proposed for aconite have been mainly of two classes (2):

(1) Those based on the reaction of the tissue of a living animal.

(2) Those based on the minimum lethal dose.

Those utilizing cats and dogs have been inaccurate; those using frogs are inapplicable, because of seasonal variations, and it has been our experience that the end-point is very indefinite. Frogs that were apparently dead within fifteen minutes after injection in ventral lymph sac were found on examination to still be just alive as long as six to eight hours later. The heart beat was apparently arrested, but upon slight stimulation, such as gentle stroking with the finger, or a slight electrical shock, it would again be set into motion and beat for some time without further stimulation. Those tests using mice have not been sufficiently experimented with and there is a wide variation in the susceptibility of rats to aconitine (3). The guinea-pig method has been highly recommended because of its sensitivity and constancy.

The chief objections to the guinea-pig method are that it is expensive, and that it is a measure of the toxicity of aconite rather than of any particular pharmacological action. It has generally been assumed that a physiological reaction such as emesis is a manifestation of the pharmacological action of the drug; therefore, an assay based on such an action is a satisfactory measure of the pharmacological activity.

EXPERIMENTAL

Part I

To determine if aconitine is the essentially active analgesic agent, it was decided to compare the analgesic activity of the other alkaloids present, benzoylaconine and aconine, and of the total alkaloids of aconite, with the analgesic activity of pure aconitine. Aconitine as purchased from Merck labeled "Aconitine Potent, 10 to 15 times strength of Amorphous" which gave a sharp melting point at 192–193° which agreed with the accepted melting point of pure aconitine (195° C.) closely enough to be accepted as pure for pharmacological work was used. This gave an even higher melting point than did our sample of U. S. P. Reference Aconitine. An attempt was made to prepare from this benzoylaconine and aconine which are breakdown products of aconitine by hydrolysis. Although many hours were spent in this attempt, and although the procedures as found in the literature were followed in every detail, benzoylaconine and aconine were never obtained in anything near the pure state.

Since this procedure did not give the desired results another method was attempted. It appeared that if aconitine was the active analgesic agent, a comparison of the analgesic action could be made between pure aconitine, the aconitine breakdown products and the total alkaloids of aconite.

The breakdown products were prepared by boiling gently a solution of aconitine with dilute ammonia water until there was a slight color change indicating a breakdown of the aconitine. It was then heated to dryness on a water-bath and placed in a desiccator over phosphorous pentoxide. This product will hereafter be referred to as the "breakdown products;" however, it is in truth a mixture of the "breakdown products and aconitine."

The total alkaloids were obtained from an "Extract of Aconite" furnished for this research by Mr. John T. Lloyd of the John T. Lloyd Laboratories of Cincinnati. It was labeled "Aconite—First Extraction." The extract was shaken out with ether and chloroform by the usual method, and the ether and chloroform volatilized and the residue dried over phosphorous pentoxide.

Since there is no known method of definitely measuring analgesia, we decided to use several of the common methods rather than to rely upon the results obtained from any one method. The methods used were:

1. The effect upon corneal reflex.
2. The effect upon frog reflex.
3. The sensitivity of the cat's tail.

In the first method rabbits were used. Varying concentrations of aconitine, breakdown products and total alkaloids were tested in this manner, but all three in dilutions even as high as 1 to 10,000 produced such irritation and extreme salivation that the results could not be taken as indicative. While the

reflex was abolished in all cases, no reliable data could be obtained.

In the second method, the frogs' reflexes were tested to 0.1% HCl and to the prick of a dissecting needle. The acid was washed off well each time. Then one leg was immersed in the solution to be tested for two minutes while the other leg was likewise immersed in the control solution. The reflex of each leg was again tested with 0.1% HCl and the prick of a needle. It was soon found that, if the reflex to the acid was abolished, it was also abolished to the needle, so for the remainder of the tests only the acid was used. The results with this method were more satisfactory. They are as follows:

Table I.—Effect of Aconitine on Frog Reflex

Dilution	Reflex to 0.1% HCl	
	Control Leg	Test Leg
1:500	Sharp	Abolished
1:2000	Sharp	Abolished
1:4000	Sharp	Abolished
1:5000	Sharp	Abolished

Table II.—Effect of Breakdown Products on Frog Reflex

Dilution	Reflex to 0.1% HCl	
	Control Leg	Test Leg
1:500	Sharp	Abolished
1:2000	Sharp	Abolished
1:4000	Sharp	Very slight (delayed)
1:5000	Sharp	Good

Table III.—Effect of Total Alkaloids on Frog Reflex

Dilution	Reflex to 0.1% HCl	
	Control Leg	Test Leg
1:500	Sharp	Abolished
1:2000	Sharp	Abolished
1:4000	Sharp	Fair
1:5000	Sharp	Good

These results indicate that aconitine is the active analgesic agent, it being more active than either the breakdown product mixture or the total alkaloids. The 1:4000 dilution of the breakdown solution did not produce complete analgesia, and the 1:4000 dilution of the total alkaloids produced practically no analgesia, but complete analgesia was still obtained with the 1:5000 dilution of aconitine.

The third method was a variation of that used by Eddy (4). The weight required to produce a definite reaction on the cat, characterized by a cry of pain and an effort to pull away, was determined on the normal cat and then the weight required to produce this same reaction on the cat after its tail had been immersed in the testing solution for a period of five minutes was determined and a comparison made.

In this experiment, only the 1:500 dilutions of each the aconitine, the breakdown products and the total alkaloids were used. Alcoholic solutions were used to facilitate absorption through the skin. It was found that the aconitine solution was about twice as analgesic as were the breakdown products and the total alkaloids; in other words, the increase in weight necessary to produce the characteristic reaction after immersion in aconitine was on the average twice the increase necessary to produce the reaction after immersion in the breakdown products or the total alkaloids. The latter two solutions produced approximately the same degree of analgesia in this experiment.

Of course, the very nature of these experiments necessitated the use of a great many test animals and it was necessary to repeat the tests many times before any conclusions could be drawn. It is realized that these tests are crude and at their best are only relative, but they do appear to indicate that aconitine, if not the only, is the chief analgesic agent in aconite.

It therefore appears that an assay of aconite could be based upon aconitine content.

Part II

Varying doses of the aconitine, aconitine breakdown products and total alkaloids of aconite were injected into a series of pigeons to determine the minimum emetic dose of each of these preparations. Solutions were used containing 0.03 Gm. per 1000 cc. in physiological salt solution.

The method used was similar to the one proposed by Hanzlik (5) for the standardization of digitalis by the pigeon emesis method and the one by Christensen and McLean (6) for the assay of *Veratrum Viride*. Heretofore most of the procedures suggested that the pigeons be starved for approximately twelve hours prior to injection. However, we obtained better results by starving the pigeons for only four to six hours. It was noticed that after the pigeons had been starved for twelve hours a certain dose would cause nausea and retching with an attempt at emesis, but no actual emesis would result. But when this same dose was administered to pigeons that had been starved for only about six hours, emesis was prompt.

After injection intravenously, the birds were placed in small wire cages for observation. A period of thirty minutes was selected as the time for observation for it was found that emesis did not occur after this lapse of time. The pigeons were observed for emesis preceded by nausea. This was characterized by ruffling of the feathers, dizziness, flapping of the wings, strained swallowing, shaking of the head, downward craning movements of the neck followed by the discharge of grain and mucous secretions.

The pigeons apparently recovered from the effect of the drug within a period of a few hours; however, they were allowed to rest for a period of one week before they were again used. This period was also necessary to allow the hematoma formed by the injection to dissolve.

In order to avoid the possibility of any change in the emetic dose due to hydrolysis of the preparations, or for any other reasons, fresh preparations were made each day immediately preceding the

injections The drug was accurately weighed on an analytical balance and the dilution made in a volumetric flask to avoid any possibility of error there.

Table IV.—Showing Average Minimum Emetic Dose of Aconitine

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.030[a]	10	..	10	..
0.031	3	..	3	..
0.032	1	..	1	..
0.034	1	1	..	..
0.036	1	..	1	..
0.037	1	..	1	..
0.038	5	2	3	..
0.039[b]	7	4	1	2
0.040	1	..	..	1
0.042	1	..	..	1
0.045	1	1	..	..

[a] Including 0.030 and less than 0.030 mg. per Kg.
[b] Minimum emetic dose.

Table V.—Showing Average Minimum Emetic Dose of Aconitine Breakdown Products

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.040[a]	9	..	9	..
0.043	3	1	2	..
0.045	1	..	1	..
0.047	5	..	5	..
0.048[b]	10	7	2	1
0.049	3	1	..	2
0.050	2	..	..	2

[a] Including 0.040 and less than 0.040 mg. per Kg.
[b] Minimum emetic dose.

Table VI.—Showing Average Minimum Emetic Dose of Total Alkaloids of Aconite

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.040	1	..	1	..
0.041	2	..	2	..
0.043	3	..	2	1
0.044	2	..	2	..
0.046	5	2	3	..
0.047[a]	9	6	2	1
0.048	2	..	..	2
0.049	2	1	..	1

[a] Minimum emetic dose.

Table VII.—Showing Average Minimum Emetic Dose of Aconitine Intraperitoneally

Dose Mg./Kg.	Number of Injections	Results of Injections: Emesis	No Emesis	Death
0.040	1	..	1	..
0.060	1	..	1	..
0.065	1	..	1	..
0.075	4	1	3	..
0.076[a]	8	6	2	..
0.077	3	..	..	3

[a] Minimum emetic dose.

For the dual purpose of comparing methods of administration and to ascertain whether the emetic action of aconitine is central or peripheral, one preparation, aconitine, was administered intraperitoneally and the minimum emetic dose determined.

In this experiment, by minimum emetic dose (M. E. D.) is meant: the intravenous dose given in solution and expressed in terms of mg. per Kg. of pigeon which will just cause emesis in two out of three pigeons.

Tables IV, V, VI and VII show the data obtained in this study.

DISCUSSION AND CONCLUSIONS

The results from this work indicate that aconitine is the active analgesic agent in aconite. It is further shown that aconitine will consistently produce emesis and is in all probability the only emetic principle in aconitine.

It appears that the determination of the minimum emetic dose of an aconite preparation would be a direct measure of the aconitine content.

In this work approximately one hundred injections were made and emesis was produced in a good percentage of them. This is contrary to the conclusions drawn by Hanzlik (5) that aconite does not produce emesis when injected intravenously. It agrees, however, with Eggleston and Hatcher (1) who produced emesis upon the intravenous injection of aconitine in dogs with a dose of 0.035 mg. of aconitine per Kg. of dog.

The technique of administration is convenient and simple and does not necessitate the help of an assistant.

For the purpose of comparing methods of administration and also to ascertain whether the emesis was due to central or peripheral action, the emetic dose of one preparation, aconitine, was determined by intraperitoneal injection. This method of administration was found to require approximately twice the dose to produce emesis as did the intravenous method of administration. It also required a much longer period of time to produce this reaction.

No other attempt was made to determine the seat of emetic action; however, several of these facts taken together indicate that the emesis of aconitine is the result of a central action. *First*, intravenous injection produced emesis in from two to twenty minutes while intraperitoneal injection required from forty-five to sixty minutes. *Secondly*, the intraperitoneal dose required to produce emesis is approximately twice the intravenous dose. And since it is known that the emetic centers are located in the

medulla and that aconitine does stimulate the other medullary centers, this would seem to further substantiate the theory that the emetic action of aconitine is central.

All the pigeons upon injection reacted in the same characteristic manner whether death followed, or whether emesis followed or whether there were no other results. Immediately after injection the bird appeared to be very ill, there was a downward drooping of the wings, dizziness and very rapid breathing. The bird's sense of equilibrium appeared to be upset for it had no control of balance and would either squat clear down until its body rested on the floor of the cage or else lean against the side of the cage.

If emesis followed it occurred in a characteristic manner as before described. If death followed it too occurred in a characteristic manner. Just preceding death there would be a violent or convulsive flapping of the wings, the bird would fall over with its neck bent far backward, and gasping for breath. Death followed in a very few seconds from what appeared to be respiratory paralysis. Occasionally death occurred before the bird could be released from the operating table, and a few times even before the needle was withdrawn.

It would appear that the chief disadvantage of the pigeon emesis method of assay is that the emetic dose is entirely too close to the fatal dose, for too many times the same dose would one time cause emesis and the next time cause death. This is further seen in the fact that in each case, regardless of the method of administration, an increase of approximately 0.001 mg./Kg. over the M. E. D. would be fatal, indicating that the emetic effect is obtained only when the near lethal dose is administered.

It was noticed that the birds which were able to vomit appeared to recover from the effects much more rapidly than did the ones that did not vomit. And also if the bird did vomit it was more likely to recover from the larger doses than if it did not vomit, for in no instance did a bird die after emesis, regardless of the size of the dose.

The results of this work indicate that the pigeon emesis method of assay is applicable to aconite and offers a possible method of standardization. It is, of course, realized that this work is for the most part only preliminary and that fruther research is necessary before any definite conclusions can be drawn.

It should also be kept in mind that this work was pursued with the intent of finding a method of standardizing the *analgesic* agent in aconite and not with any reference to the cardiac or blood pressure effects.

The pigeon emesis method of assay is simple, rapid, economical, has a definite and unmistakable end-point and gives a reasonable degree of accuracy.

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Assay of Digitalis

The Use of Dogs in, and a Comparison of the International Standard (1936) and the U. S. P. Reference Powder

*By Philip Blickensdorfer and H. A. McGuigan**

In this work we present evidence to show that dogs are quite suitable animals for the standardization of digitalis; that the U. S. P. Digitalis Reference Powder is stronger than it is labeled; and to correct a measurable error in the data of a previous paper.[1]

EXPERIMENTAL

Method.—The method used is that described by McGuigan and McGuigan (1). Dogs are anes-

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[1] Paper by McGuigan and McGuigan, *J. Pharm. and Exp. Therap.*, 63 (1938), 76.

thetized by intraperitoneal injection of 35 mg. per Kg. of pentobarbital in water solution. In about 15 minutes, they are ready for digitalis injections. The tincture of digitalis (0.1 cc. per Kg.) is injected slowly (at least 1 minute for each injection) into the femoral vein. Injections are repeated every five minutes until the animal dies. If the tincture of digitalis is of International Standard strength, twelve injections will stop the heart.

Test of the Method on Commercial Products.—To compare our results on the dog with those obtained by using the frog, we asked five leading manufacturers of digitalis preparations to furnish us with samples and their data on standardization. All gladly complied with our request. We found all the samples submitted to comply with the U. S. P. standards, but with considerable variations. One house sent us an old sample (I), which they had retested, and our results agreed exactly with their work. The average results from closely agreeing individual experiments are as follows:

Table I.—Results of Test of Method on Commercial Products

Sample	Our Findings	Manufacturer's Statement
I	86%	85%
II	83%	100%
III	111%	111%
IV	120%	110%
V	92.5%	100%
Average	98.5%	101%

We believe these results show the method to be quite dependable, since the slight differences are well within the limit of experimental error.

DISCUSSION

Unsolved Questions.—While we believe that the use of the dog as we have outlined in the standardization of digitalis gives consistent and reliable results, there are some factors that future work may improve. The first of these is the anesthetic. Pentobarbital in our experience does not always give the same depth of anesthesia. When the anesthesia varies, as sometimes happens, the blood pressure, after a few injections of digitalis, may rise to enormous heights, occasionally to 260 or even 300 mm. of mercury. It has been shown that the kind of anesthesia, which we think means the depth of anesthesia, changes the fatal dose (2).

The effect on the vasomotor center especially needs investigation. When the pressure is high, digitalis may kill with smaller doses. This is a factor concerned in many of our lower variations from the average. Again, in cases where the blood pressure remains low throughout the experiment, more digitalis is required to stop the heart. This accounts for some of the highest doses we have recorded.

There is some relationship between these wide variations and oxygen supply, though we do not think this explains all. However, we have been able to revive animals, apparently dead, with artificial respiration, so that one or two more injections were required to kill the heart. Quite often this "asphyxia (?)" occurs at the point where the toxic action on the heart begins, especially if it occurs rapidly. In such cases, apparently, depression of the respiratory center is a complicating factor. A study of these factors will improve the method, but biological standardization will never have the accuracy of a chemical balance.

The Lag of Digitalis Action.—The question has arisen, how much, if at all, the last one or two injections are active in causing death, because they may not have time to act. We believe that this factor is not so important because:

1. The conditions are the same in the standardization of any sample as they are in the control.

2. We are able to show that even the first injection has an unseen action within five minutes. If, before injecting digitalis, one tests the rise given by an injection of adrenalin, and then injects digitalis, a second injection of adrenalin within a five-minute period gives a more marked rise. That an action on the heart occurs after the first injection is shown by the fact that after two or three injections of digitalis an injection of adrenalin immediately elicits the slowing effect of digitalis on the heart, which would not arise normally until more digitalis is given. However, when the heart is damaged to such an extent that the blood pressure becomes very low, as happens sometimes near the end of the experiment, there may be some lag in the last one or two doses. Consequently, if the blood pressure becomes so low that circulation is impeded, it may be hard to decide whether the last dose is effective.

Again the selection of dogs may be important. We have used dogs as they were

brought in from the pound. The only requirement was that they should be in a "fair" state of health. No "conditioning" was attempted though we believe by more critical selection more closely agreeing results may be obtained. Chows, in our experience, are unreliable. To avoid bias, we include all our results, although some, quite wide from the average, might justifiably be omitted.

EXPERIMENTAL

A tincture was prepared by macerating the equivalent of 8 Gm. of the powder in 100 cc. alcohol (4 volumes of alcohol and 1 volume of water), as directed by the U. S. P. XI. One cubic centimeter of this tincture is equal to one International Unit. The results obtained are shown in Table II.

Table II.—Results with a Tincture Prepared with the International Standard (1936) Powder

Weight of Dog, Kg.	Number of Injections	Dose, mg. per Kg. (Corrected)	International Units per Kg.
6.4	15	150	1.5
8.6	13	130	1.3
10.0	12	120	1.2
6.4	15	150	1.5
8.6	15	150	1.5
10.9	13	130	1.3
7.7	10	100	1.0
4.9	12.5	125	1.25
13.2	12	120	1.2
9.6	12	120	1.2
14.5	10.5	105	1.05
12.0	11	110	1.1
8.2	11	110	1.1
8.2	13	130	1.3
Average	12.5	125	1.25

Table III.—Results with Tincture Prepared with U. S. P. Reference Powder (0.745 Gm. = 1 Gm. International Strength) in Dogs

Weight of Dog, Kg.	Number of Injections	Dose, mg. per Kg. (Corrected)	Units per Kg.
11.4	9	90	0.9
9.6	13	130	1.3
18.2	9	90	0.9
10.0	9	90	0.9
7.7	10	100	1.0
6.4	11	110	1.1
14.0	8	80	0.8
14.5	9.5	95	0.95
14.5	9	90	0.9
12.7	10	100	1.0
9.3	10	100	1.0
12.8	10	100	1.0
Average	9.8	98	0.98

Table III shows the results obtained when a tincture was prepared by macerating the equivalent of 7.45 Gm. of the powder in 100 cc. of alcohol for 24 hours, and the supernatant liquid obtained by centrifugation used; 0.1 cc. per Kg. was injected every 5 minutes.

Table IV.—Results with Tincture Prepared with U. S. P. Reference Powder (0.745 Gm. = 1 Gm. International Strength) in Cats

Weight of Cat, Kg.	Number of Injections	Fatal Dose, mg. per Kg. (Corrected)	Units per Kg.
1.8	11	110	1.1
2.6	6	60	0.6
3.9	13	130	1.3
2.1	14	140	1.4
2.6	10	100	1.0
2.3	11	110	1.1
2.5	7	70	0.7
2.6	9	90	0.9
3.4	10	100	1.0
1.7	7	70	0.7
Average	9.8	98	0.98

While the average is 98 mg. per Kg. in both dogs and cats, the outstanding figure is 100 mg. per Kg. No artificial respiration was used on these cats, and we think some of the low figures in cats may be complicated with respiratory failure. The average dose for cats is practically the same as for dogs, *i. e.*, 0.1 cc. per Kg., but the variation is wider. However, from the work below and the results of other workers, some doubt exists as to this dosage for cats. The work of Bone, Elam and Blickensdorfer in the following paper (see page 105) justifies this opinion.

If we take the values found for dogs, 120 mg. per Kg. (1.2 units) for the International Standard, and 100 mg. per Kg. for the U. S. P. Reference Powder, then the reference powder is about 20 per cent stronger than it is labeled. The label states that 0.745 Gm. = 1 Gm. of International Powder. If 0.745 = 120 per cent, 100 per cent = 0.62 Gm. We, therefore, made a tincture using 0.62 Gm. to 10 cc. alcohol and tested it on dogs with the following results:

Table V.—Results with Reference Powder in Dogs (Factor 0.62 Instead of 0.745)

Weight of Dog, Kg.	Number of Injections	Fatal Dose, mg. per Kg. (Corrected)	Units per Kg.
8.8	13	130	1.3
13.6	15.5	155	1.55
7.7	10	100	1.0
10.2	12.5	125	1.25
9.6	11	110	1.1
10.0	12.5	125	1.25
12.0	13	130	1.3
Average	12.5	125	1.25

The tincture referred to above (made by using 0.62 Gm. of the U. S. P. Reference Powder to 10 cc. of alcohol) was also tested on cats with the results shown in Table VI.

Table VI.—Results with Reference Powder in Cats (Factor 0.62 Instead of 0.745); Artificial Respiration Used

Weight of Cat, Kg.	Number of Injections	Fatal Dose, mg. per Kg. (Corrected)	Units per Kg.
3.3	10	100	1.0
3.4	13	130	1.3
3.0	8	80	0.8
3.4	12	120	1.2
3.2	10	100	1.0
Average	10.6	106	1.06

With five cats with the International Powder, we found an average of 1.02 cc. of the tincture per kilo.

DISCUSSION

These results show that when we use dogs in the standardization of digitalis, the reference powder is 20 per cent stronger than it is labeled. By using cats, less consistent results are found, the reason for this being that cats are more variable in their reaction.

From work on frogs, both by the one-hour method and the minimum lethal dose method, Rowe (3) concludes that the U. S. P. XI Reference Powder is about 25 per cent stronger than labeled.

Spruth and Olsen (4), also using frogs, found that the U. S. P. Reference Powder varied from 110 to 130 per cent of the International Standard.

Edmunds and co-workers (5) found the U. S. P. Reference Powder, in terms of the International Standard (100 per cent) to be by the cat method, 141.65%; one-hour frog method, 134.22%; four-hour frog method, 157.37%; twelve-hour frog method, 190.40%. The average of all frog methods is 160.66, or exactly as we have found in the dog. In our terms, 100/62 = 161 per cent. Our results are also practically the same as his results on the four-hour frog method.

The lower strength shown by the one-hour method seems due to less absorption. Lessened absorption or irregularities in absorption lessen the value of this method.

Supposing we have two samples of digitalis, exactly 80 and 100 per cent, to show this difference by the frog method, it will be necessary that each be absorbed to the same degree. If 50 per cent of each is absorbed in one hour, 50 per cent of 100 = 50, and 50 per cent of 80 = 40, the difference is 20 per cent. However, if 50 per cent of the stronger is absorbed (50), and 25 per cent of the weaker (20), the difference is 60 per cent. Again, if 50 per cent of the weaker (40) and 25 per cent of the stronger (25) is absorbed, the difference is 37.5 per cent; but the stronger sample is to be only 62.5 per cent that of the weaker. Consequently irregularities of absorption are potential factors of enormous error. The four-hour method or longer would lessen this error.

In the work of McGuigan and McGuigan, while they found that an average of 12 injections (120 mg. per Kg.) of the reference powder kills dogs, they prepared their solutions by making them up to volume by washing the residue. The U. S. P. method uses only the supernatant liquid. This error is measurable because it is quite difficult to wash the powder free from the active ingredient. Their results are correct for the International Standard, and for what the reference powder should be.

CONCLUSIONS

1. Dogs are reliable animals for the standardization of digitalis.

2. The dose of International Powder in the form of the tincture, prepared as directed by the U. S. P. XI and using only the supernatant fluid, is (corrected) 120 mg. (1.2 units) per Kg., or an average of 12 injections of 0.1 cc. per Kg., given intravenously.

3. The U. S. P. Reference Powder is 20 per cent stronger than labeled. Instead of 0.745, the factor should be 0.62.

To determine the dose for cats more satisfactorily we refer to the accompanying paper by Bone, Elam and Blickensdorfer.

We thank J. A. Vaichulis for valuable assistance in this work; Abbott Laboratories, Eli Lilly and Company, Merck and Company, Parke, Davis and Company and E. R. Squibb and Sons for coöperation; also the Trustees of the U. S. P. for supplying Reference Powder and International Standard.

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Titles of papers to be presented at the Richmond meeting must reach Secretary E. F. Kelly by March 18th to be included in the printed program.

The Cat and Dog Units of Digitalis

*By J. A. Bone, J. W. Elam and Philip Blickensdorfer**

Blickensdorfer and McGuigan, by the use of dogs, found the U. S. P. 1938 Digitalis Reference Powder to be 20 per cent stronger than labeled. Their results with cats were inconsistent and required more work before definite conclusions could be drawn regarding the dose for cats. The object of this work is to determine more accurately the cat dosage.

In their short series of experiments with cats, Blickensdorfer and McGuigan (Table III of their article, see page 103 of THIS JOURNAL) found that the dosage of the reference powder for the dog was 1.2 cc. per Kg. body weight. However, the results with cats showed too wide an individual variation to be acceptable. Part of this variation they think is due to the high concentration of the reference powder. In any biological standardization, an unaccountable variation of 10 per cent or more may occur. If the preparation is stronger than standard, the variations may be greater. When they used the factor 0.62 instead of 0.745, their dosage for cats was less than the dosage for dogs. However, the number of cats used (5) is insufficient to accept their results as the cat unit. In this work we have used enough cats to justify a comparison of the cat dosage with that for dogs.

EXPERIMENTAL

Method Used.—Cats were anesthetized with pentobarbital, 35 mg. per Kg. intraperitoneally. The tincture of digitalis was injected slowly into the femoral vein at the rate of 0.1 cc. per kilo every five minutes, until the heart stopped. Artificial respiration was used throughout. The tincture of digitalis was prepared by using the ratio of 0.62 Gm. of the reference powder, macerated for 24 hours, in 10 cc. of 70 per cent alcohol. The alcohol in this dose has little effect on the heart and kills by action on the respiratory center. By actual experiment we have found that at least four times the volume of alcohol is necessary to kill, and then by action on the respiratory center.

We used about an equal number of male and female animals, without noticeable difference in the results. The animals were all in an excellent state of health, and were not kept in captivity after bringing to the laboratory. We think incarceration except under rare conditions may mitigate against uniform results.

The following table shows the complete data:

Table I.—Results with a Tincture Prepared with U. S. P. Digitalis Reference Powder (0.62 Gm. in 10 Cc. 70% Alcohol)

Wt. of Cat, Kg.	No. of Injections	Total Dose, mg. per Kg. (Corrected)	Units per Kg.
3.3	10	100	1.00
3.4	13	130	1.3
3.0	8½	80	0.8
3.4	12	120	1.2
3.2	10	100	1.0
2.6	8	80	0.80
3.0	10	100	1.00
2.3	10	100	1.00
2.3	12	120	1.20
3.1	11	110	1.10
2.3	10	100	1.00
2.8	10	100	1.00
2.7	11	110	1.10
2.0	10	100	1.0
3.1	12	120	1.2
3.7	11	110	1.1
2.85	11	110	1.1
2.2	9	90	0.90
3.6	10	100	1.0
2.6	6	60	0.6
3.0	8	80	0.8
2.5	12	120	1.2
4.2	9	90	0.9
3.1	9	90	0.9
2.6	12	120	1.2
Average	10.16	101.6	1.016

Table II.—Results with a Tincture Prepared with the International Powder (0.8 Gm. in 10 cc. 70% Alcohol)

Wt. of Cat, Kg.	No. of Injections	Total Dose, mg. per Kg. (Corrected)	Units per Kg.
2.7	9	90	0.90
1.9	11	110	1.10
3.3	9	90	0.90
3.2	10	100	1.00
2.5	12	120	1.20
Average	10.2	102	1.02

SUMMARY AND CONCLUSIONS

The results with cats, as with dogs, support the opinion that the U. S. P. Digitalis Reference Powder is twenty per cent stronger than labeled. Instead of the factor of 0.745, the strength found is 0.62. Using this factor our results with cats are in harmony with previous workers and the average found is almost "ideal" for a dose of 1.00 cc. per Kg. weight of cat. Almost any sequence of five cats gives accurate results and within 10 per cent of the average dose. However, much of the idealism vanishes, when we note that the range varies between

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six and thirteen injections. Yet the average which must be taken is good, and the majority of the findings are close to the average. The method we present is simpler than that presented by others, in that the tincture may be used directly. The alcohol is not an interfering agent, since it kills, not by action on the heart but by action on the respiratory center, and several times the volume contained in the tincture is required to kill. Pentobarbital, as we have used it, is as good an anesthetic for cats, as for dogs.

In terms of the International Standard, the U. S. P. Reference Powder is 80/62 = 129% or 29 per cent stronger.

The experiments recorded in this paper and that by Blickensdorfer and McGuigan show:

1. That by the use of both dogs and cats, the U. S. P. Reference Powder is 20 per cent stronger than it is labeled. Instead of the factor 0.745, the factor 0.62 is more nearly correct.

2. When a tincture is prepared using 0.62 Gm. of the reference powder in 10 cc. alcohol, or 6.2 Gm. in 100 cc., the dose for dogs is 1.2 cc. per Kg. body weight. The dose for cats is 1.0 cc. per Kg.

3. The results are in harmony with the Hatcher dose for the cat, and agree closely with the results reported by other investigators using the frog method.

We wish to thank the Board of Trustees of the United States Pharmacopœial Convention for furnishing the Digitalis Reference Powder and the International Powder. We express our thanks to Professor E. Fullerton Cook for his courtesy and coöperation.

NOTICE

The next annual meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION will be held in Richmond, Va., May 5th to 12th.

The Action of Ephedrine on Halogenated Organic Compounds*

By Frank A. Steldt and K. K. Chen

The fact that ephedrine base reacts with chloroform to give ephedrine hydrochloride was first pointed out by Peterson in 1927 (1). To date no other organic compounds have been reported to have a similar reaction with ephedrine, although *a priori* it would seem possible. In order to test the validity of this idea, the present investigation was undertaken.

EXPERIMENTAL

A total of thirty-one halogenated organic compounds was examined. As shown in Tables I and II, twenty-three of the compounds reacted with ephedrine base to give the corresponding halide salt, and one, *o*-chlorobenzaldehyde, gave an addition product. The formation of the last substance is not surprising for ephedrine has been known to react with certain aldehydes, forming addition products in the proportion of one molecule of ephedrine to one molecule of aldehyde with the elimination of one

Table I.—Reaction with Ephedrine Base

Number	Name of Compound	Reaction with Ephedrine Base
1	Allyl bromide	+
2	Allyl iodide	+
3	Allyl chloride	+
4	*i*-Amyl bromide	+
5	*i*-Amyl chloride	+
6	Benzotrichloride	+
7	Benzyl chloride	+
8	Bromal	+
9	Bromoform	+
10	Carbon tetrachloride	+
11	Chloral	+
12	*o*-Chlorobenzaldehyde	+
13	β-β'-Dichlorethyl ether	+
14	Ethyl chloride	+
15	Ethylene chlorhydrin	+
16	Ethylene bromohydrin	+
17	Ethylene dichloride	+
18	Glycerol α-monochlorohydrin	+
19	*n*-Propyl chloride	+
20	Propylene dichloride	+
21	Tribromoethanol (avertin)	+
22	Tribromoethylene	+
23	Trichloroethylene	+
24	*i*-Amyl iodide	+
25	Bromobenzene	−
26	Chlorobenzene	−
27	*o*-Chlorophenol	−
28	Iodobenzene	−
29	*tert*-Butyl chloride	−
30	*o*-Chlorophenetole	−
31	Trichlorobenzene	−

* From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

Table II.—Compounds Formed with Ephedrine Base

Number	Name of Compound	Time of Reaction	M. P. of Compound Formed, ° C. (Corr.)	New Compound Formed	Remarks
10	Carbon tetrachloride	5 minutes	219	Ephedrine hydrochloride	Yellow color on standing
23	Trichloroethylene	Over night	219	Ephedrine hydrochloride	
5	*i*-Amyl chloride	5 days	218.5–219	Ephedrine hydrochloride	
13	β-β′-Dichlorethyl ether	3 hours	213 –215	Ephedrine hydrochloride	
3	Allyl chloride	15 minutes	219	Ephedrine hydrochloride	
11	Chloral	3 hours	217 –218.5	Ephedrine hydrochloride	Heat evolved
19	*n*-Propyl chloride	2 days	218 –219	Ephedrine hydrochloride	
17	Ethylene dichloride	2 days	219 –220	Ephedrine hydrochloride	
20	Propylene dichloride	2 days	219 –220	Ephedrine hydrochloride	
6	Benzotrichloride	2 hours	219 –220	Ephedrine hydrochloride	
7	Benzyl chloride	5 minutes	216 –217	Ephedrine hydrochloride	
14	Ethyl chloride	Over night	216 –217	Ephedrine hydrochloride	Sealed tube; amine odor noted on opening
12	*o*-Chlorobenzaldehyde	15 minutes	123	Addition product	
1	Allyl bromide	5 minutes	204 –205	Ephedrine hydrobromide	Much heat evolved
2	Allyl iodide	15 minutes	164 –165	Ephedrine hydroiodide	Much heat; ppt. brown, tarry
9	Bromoform	3 days	205 –206	Ephedrine hydrobromide	
22	Tribromoethylene	4 hours	206 –207	Ephedrine hydrobromide	Much heat; gas evolved
24	*i*-Amyl iodide	2 days	155 –159	Ephedrine hydroiodide	
8	Bromal	Immediate reaction	208.5	Ephedrine hydrobromide	Much heat; gas evolved; dark brown tarry ppt. in several days
21	Tribromoethanol	4 hours	206 –207	Ephedrine hydrobromide	
15	Ethylene chlorohydrin	2 days	212 –214	Ephedrine hydrochloride	Easily forms saturated soln. with ephedrine hydrochloride
16	Ethylene bromohydrin	2 days	206 –207	Ephedrine hydrobromide	
18	Glycerol α-monochlorohydrin	2 days	217 –218	Ephedrine hydrochloride	
4	*i*-Amyl bromide	4 hours	206 –207.5	Ephedrine hydrobromide	

molecule of water (2). Of special interest are the reactions of ephedrine base with chloral, carbon tetrachloride or avertin (tribromoethanol). It thus becomes obvious that ephedrine on the one hand and chloral, carbon tetrachloride and avertin on the other are incompatible in prescription writing.

The reagents used were the purest obtainable on the market. When impurities due to preparation or spontaneous decomposition were suspected, they were distilled under reduced pressure. The ephedrine base employed was the anhydrous, natural laevo-rotatory form. Each experiment was conducted as follows: Approximately 0.2 Gm. ephedrine base was placed in a test-tube and 5 cc. of the halogenated compound added. The tube was then shaken until the ephedrine went into solution, stoppered loosely and allowed to stand at room temperature for several days. Sometimes frequent shaking facilitated precipitation. The time of reaction varied greatly; in some cases precipitation was complete in five minutes and in others several days were necessary. A positive reaction usually ended in a crystalline white precipitate, although with the bromo and iodo derivatives brown gummy precipitates resulted in a few instances. Heat was evolved in several cases, accompanied by ebullition and effervescence. Upon completion of the reaction, the precipitate was filtered off and washed with ether; then recrystallized from alcohol. The melting point was deter-

mined, and for the sake of identification, a mixed melting-point determination with the respective known ephedrine halides was made.

The compound formed by the reaction of ephedrine base with *o*-chlorobenzaldehyde crystallized in colorless prisms melting at 123° C. (corrected) and had a specific rotation of $[\alpha]_D^{27} = -74.5°$ in alcohol. The substance was insoluble in water and soluble in all common organic solvents, such as ether, acetone, chloroform, ethyl acetate, benzene and alcohol. The reaction took place with great facility, 15 minutes being sufficient for its completion. That it is an addition product of ephedrine and *o*-chlorobenzaldehyde, having the empirical formula $C_{17}H_{18}ONCl$, with the loss of one molecule of water, was proved by combustion analyses as follows:

For $C_{17}H_{18}ONCl$:

Calculated	C = 70.93;	H = 6.31;	N = 4.87;	Cl = 12.33.
Found	C = 70.94;	H = 6.25;	N = 4.71;	Cl = 12.12.
	C = 70.92;	H = 6.23;	N = 4.69;	Cl = 11.88.

In consideration of the results, it becomes apparent that ephedrine readily extracts the halogens from the alkyl, allyl and substituted alkyl halides to form the corresponding halogen salt. To the last class belong the halohydrins, benzyl chloride, benzotrichloride, chloral, bromal and avertin. It fails to react with less reactive substances such as *tert*-butyl chloride. It also fails to react with the halogen atom attached to the benzene ring, although it easily attacks the same when located on the side chain of aromatic compounds. The addition compound with *o*-chlorobenzaldehyde follows a different pattern of reaction since the position of the chlorine atom is obviously not altered.

The exact mechanism of the reaction between ephedrine base and halogenated organic compounds is not yet known, although suggestive explanations may be found in the works of organic chemistry. According to Whitmore (3), (4), amines can react with allyl and alkyl halides to remove halogen acid—this property of amines being due to their basic nature. Similarly, halohydrins (5) hydrolyze rapidly in the presence of weak bases. Since our tests were not made under strictly anhydrous conditions, there was the possibility that the moisture present in the reagents and the atmosphere may have been sufficient for hydrolysis. The insolubility of the ephedrine salts in the reagents used perhaps also favors the displacement of the equilibrium toward their stability after they are formed. Further investigations will be necessary in order to determine the true mechanism of this reaction.

CONCLUSION

1. Of thirty-one halogenated organic compounds studied, anhydrous ephedrine base reacts with alkyl, allyl and substituted alkyl halides to form the corresponding ephedrine halide salt.

2. Carbon tetrachloride, chloral and avertin (tribromoethanol) are therefore incompatible with ephedrine base in prescription compounding.

3. Anhydrous ephedrine base does not react with the halogen atom that is attached to the benzene ring.

4. Anhydrous ephedrine base combines with *o*-chlorobenzaldehyde to form an addition product, $C_{17}H_{18}ONCl$, by the elimination of one molecule of water.

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The Preparation of Alkaline Bismuth Saccharates*

By G. O. Doak, Ph.D.

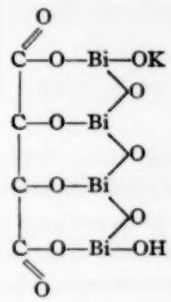
Bismuth in the form of the hydroxide or a soluble salt will dissolve in alkaline solutions of polyhydroxy acids or other polyhydroxy compounds to form solutions which vary in stability with the nature of the hydroxy compound used. In many cases the resulting bismuth complex can be precipitated from solution by the addition of alcohol. The literature contains innumerable examples of this reaction and a number of different structures have been advanced for the resulting bismuth complexes. Warren (1) has discussed the extreme variation in the different bismuth tartrates appearing on the market. Corfield and Adams (2) state that the composition of the bismuth tartrates varies with the method used for preparation. Other authors have recorded similar results with other polyhydroxy acids. Rosenheim (3) has studied these compounds and has assigned structures in

* From the Research Laboratories of George A. Breon and Co., C. W. Sondern, Ph.D., Director.

which the bismuth is linked to at least two oxygen groups of the polyhydroxy acid. Browning (4) and Maschmann (5) have assigned similar structures to the alkali bismuthyl tartrates and other alkali bismuthyl salts. Hepner and Likiernik (6) have explained these compounds by means of Werner's theory. Very little work however has been done on the experimental proof of the structure of these compounds and it may be said that as yet there is some doubt that all the bismuth complexes of polyhydroxy compounds represent true chemical compounds.

An essential feature in the preparation of these bismuth complexes is that there be two hydroxyl groups in adjacent positions to each other. Thus we have found that alkaline solutions of such glycols as ethylene glycol and 1,2-propylene glycol will dissolve large quantities of bismuth hydroxide, while 1,3-trimethylene glycol and diethylene glycol, in which the hydroxyl groups are not on adjacent carbon atoms will not dissolve bismuth hydroxide. This would support Rosenheim's structure whereby the bismuth is joined to at least two oxygen atoms on adjacent carbon atoms. The function of the polyhydroxy compounds may possibly be similar to the complexes of boric acid and polyhydroxy compounds in which two hydroxyl groups must be on adjacent carbon atoms for complex formation (10), (11). That the carboxyl group also enters into the binding of the bismuth is apparent from several facts. Alkaline bismuth complexes of glycols cannot be isolated as solid compounds. On the addition of alcohol to the solutions, the bismuth is precipitated as bismuth hydroxide leaving the glycol behind. This does not occur with the alkaline bismuth complexes of the hydroxy acids. They may be precipitated with alcohol, and redissolved in water and there is no change in their composition.

Rosenheim (3) and Browning (4) report the preparation of soluble alkaline derivatives of glycolic and malic acids. In repeating the work of these authors we have found that bismuth hydroxide will not dissolve in alkaline solutions of glycollic or malic acid, but if a soluble bismuth salt such as bismuth nitrate in acetic acid solution is warmed with an acetic acid solution of glycolic acid, a bismuthyl glycolic acid is precipitated which dissolves readily in alkali. It has been found impossible, however, to prepare bismuthyl oxalates, so that at least one hydroxyl group and one carboxyl group must be present to form a bismuthyl complex. However, there are compounds in which more than one bismuth is bound by two hydroxyl groups. Thus Rosenheim has prepared potassium tri-bismuthyl tartrate, to which the formula $K[(BiO)_3C_4H_2O_6].4H_2O$ has been assigned. Kober (7) has prepared a tetra-bismuth tartrate to which he assigned the formula



Such a formula appears to be unlikely since one bismuth would be functioning as both an acid and base at the same time. Lauter and Braun (8) have prepared both di- and tri-bismuthyl gluconates but failed to obtain a tetra-bismuthyl gluconate.

Rosenheim (3) also succeeded in preparing in crystalline form mono-potassium di-bismuthyl saccharate with the formula $K[Bi_2(C_6H_{10}O_{16})]$ while Browning (4) prepared a di-bismuthyl saccharic acid and its sodium salt. We have prepared a di-potassium di-bismuthyl saccharate and a di-potassium tri-bismuthyl saccharate. Careful acidification of the di-bismuthyl compound gave di-bismuthyl saccharic acid which was apparently identical with Browning's compound. We could not prepare stable di-sodium or di-ammonium salts of this acid as they tended to hydrolyze in water. Further we could not prepare a potassium bismuthyl saccharate with more than 3 atoms of bismuth per mole of saccharic acid, corresponding to Kober's potassium or sodium tetra-bismuthyl tartrate. While the structure of the bismuth complexes with

polyhydroxy compounds is still an unsettled question, in view of the results reported in this paper, the formulas suggested by Rosenheim (3) seem to best satisfy the properties of the alkaline bismuth saccharates and the following formula is tentatively suggested for di-potassium di-bismuthyl saccharate:

Such a formula then explains why the polyhydroxy alcohols dissolve bismuth hydroxide but the presence of a carboxyl group is necessary for their isolation in the solid state. It also explains our failure to obtain a potassium bismuthyl saccharate containing more than three moles of bismuth per mole of saccharic acid; the failure of polyhydroxy alcohols without hydroxyl groups on adjacent carbons atoms to dissolve bismuth hydroxide; and the instability of the di-potassium tri-bismuthyl saccharate, since in this compound the third bismuth atom must be linked with the two center hydroxyl groups and the carboxyl groups contribute little or nothing to the stability of this linkage. Both the potassium di- and tri-bismuthyl saccharates are white solids, soluble in water, insoluble in organic solvents. Attempts to recrystallize them from water or water-alcohol mixtures were unsuccessful. They were purified by reprecipitating several times with alcohol from aqueous solution. On redissolving the di-bismuthyl saccharic acid in potassium hydroxide and precipitating with alcohol there was no appreciable change in bismuth content from the original potassium di-bismuthyl saccharate, so it would seem probable that the compound was of uniform composition. In solution the potassium di-bismuthyl saccharate appears to be less affected by light than the corresponding tartrate and gluconate. On exposing ampuls of solutions of these compounds to light metallic bismuth is precipitated; most rapidly by the tartrate, slowly by the gluconate and hardly at all by the saccharate. Potassium di-bismuthyl saccharate forms a solution which appears to be stable indefinitely and consequently it is of interest as a drug for the treatment of those diseases for which bismuth is used. Von Oettingen, Sollmann and Schweid (9) have shown that sodium bismuthyl tartrate precipitates rapidly with cattle serum, while sodium bismuthyl citrate precipitates much more slowly under the same conditions. These authors suggest that the immediate toxic effect of these compounds on injection is due to this precipitation. In repeating these experiments with both horse and human serum we have found an immediate precipitate with sodium bismuthyl tartrate, which became progressively heavier on standing, a faint cloudiness after several hours with potassium bismuthyl gluconate and no precipitation or cloudiness even after three days with potassium bismuthyl saccharate.

EXPERIMENTAL

Di-Potassium Di-Bismuthyl Saccharate.—Seventeen cubic centimeters of a 60% solution of saccharic acid (or 12.4 Gm. of pure potassium acid saccharate) ($^1/_{10}$ mole) were dissolved in 250 cc. water and just neutralized with KOH, and 10 cc. of 25% KOH added in addition. Bismuth hydroxide, freshly precipitated from 48.5 Gm. ($^1/_{10}$ mole) of bismuth nitrate and suspended in 500 cc. of water were added slowly over several hours to the potassium saccharate solution. The reaction was stirred vigorously during the addition. When all the bismuth hydroxide had dissolved, the solution was filtered and the bismuth complex precipitated with 95% alcohol. This was redissolved and reprecipitated three times, washed with alcohol and then with ether and dried *in vacuo* at 100°. The yield was 35 Gm.

Calculated for $C_6H_6H_{10}Bi_2K_2.H_2O$: Bi = 55.58; K = 10.40; C = 9.58; H = 1.07.
Found: Bi = 56.75%; K = 8.58%; C = 9.76%; H = 1.65%.

Di-Bismuthyl Saccharic Acid.—Ten grams of potassium di-bismuthyl saccharate dissolved in water were precipitated by the cautious addition of 10% hydrochloric acid. The precipitate was

washed free from chlorides, then washed with alcohol and ether and finally dried *in vacuo*.

Calculated for $C_6H_8O_{16}Bi_2$: Bi = 63.53%.
Found: Bi = 63.50%.

Sodium Di-Bismuthyl Saccharic Acid.—An attempt to prepare sodium di-bismuthyl saccharate by the same procedure used for the preparation of potassium di-bismuthyl saccharate yielded a white solid which after precipitation with alcohol was no longer soluble in water. The sodium salt, however, was made directly from di-bismuthyl saccharic acid. Ten grams of the acid as prepared above were suspended in water and 10% sodium hydroxide added until the acid just dissolved. This was then precipitated with alcohol three times as in the preparation of the potassium compound, washed with alcohol and ether and dried *in vacuo*. Analyses indicated however that the product was the acid salt.

Calculated for $C_6H_7O_{10}Bi_2Na.H_2O$: Bi = 59.89; Na = 3.29.
Found: Bi = 61.48; Na = 3.60.

Sodium Potassium Di-Bismuthyl Saccharate.—Twelve and four-tenths grams ($^1/_{20}$ mole) of potassium acid saccharate were just neutralized with sodium hydroxide and then 25 cc. excess of 25% sodium hydroxide added. To this was added freshly precipitated bismuth hydroxide from 48.5 Gm. ($^1/_{10}$ mole) of bismuth nitrate and the product precipitated with alcohol and purified in the same manner as the di-potassium compound. The resulting white compound was dried *in vacuo*.

Calculated for $C_6H_7O_{10}Bi_2NaK.H_2O$: Bi = 56.80; Na = 3.12.
Found: Bi = 59.14; Na = 3.32.

Di-Potassium Tri-Bismuthyl Saccharate.—Seventeen cubic centimeters of a 60% solution of saccharic acid ($^1/_{20}$ mole) were just neutralized with potassium hydroxide and 50 cc. of 25% solution added in excess. To this was added freshly precipitated bismuth hydroxide from 72.7 Gm. ($^3/_{20}$ mole) of bismuth nitrate in 200 cc. of water. The mixture was shaken for 48 hours as described by Kober (7), filtered and the bismuth complex precipitated with alcohol and purified in the same manner as the previous compounds. The resulting white compound appeared to be less stable than the di-bismuthyl compounds and turned brown when warmed *in vacuo*. It was dried at room temperature over calcium chloride.

Calculated for $C_6H_9O_{11}Bi_3K_2.2H_2O$: Bi = 63.06; K = 7.86.
Found: Bi = 63.13; K = 7.05.

An attempt was also made to prepare a compound containing 4 atoms of bismuth per mole of saccharic acid using the same procedure as in the preparation of the tri-bismuthyl saccharate but with a large excess of bismuth hydroxide. The resulting compound contained only 63% bismuth. It is apparently impossible to prepare potassium bismuthyl saccharates with more than 3 atoms of bismuth per mole of saccharic acid.

SUMMARY

1. Methods are described for preparing di-potassium di-bismuthyl saccharate, di-bismuthyl saccharic acid, mono-sodium di-bismuthyl saccharate, sodium potassium di-bismuthyl saccharate and di-potassium tri-bismuthyl saccharate.

2. Potassium bismuthyl saccharate has been shown to be a more stable complex than the corresponding tartrate and gluconate.

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Studies on Cantharides

II. The Assay of Cantharides*

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INTRODUCTION

It is generally acknowledged that the present pharmacopœial process for the assay

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of Cantharides is not without fault. It is time-consuming and does not give uniform results. Extraction of cantharidin from the crude drug is incomplete with some solvents. The volatility of cantharidin has also received too little consideration. Although cantharidin has a high melting point (218°) it is easily volatile at lower temperatures and Reimers (1) has shown losses to occur upon drying above 60°. It is even more volatile in the presence of solvent vapors.[1] Finally, the method of purification is faulty; purification of the extracted cantharidin by the official process is incomplete and the final residue which is weighed does not accurately represent the amount of cantharidin extracted.

A colorimetric determination (2) of cantharidin has not been possible; likewise, a distillation method (3) was no more successful. The idea of applying a titrimetric method to the estimation of cantharidin is not a new one, but has not been realized because it has not been shown that cantharidin is capable of being titrated. With the successful titration of this compound, as reported in the previous paper (4), it was now possible to attempt an application of this method in the improvement of the present official assay process. Since extraction, evaporation and purification procedures must still be dealt with in any method, it seemed desirable to study also these aspects of the problem.

EXPERIMENTAL

Determination of Cantharidin.—By U. S. P. XI Method: A sample of Cantharides, labeled Russian Cantharides, was obtained from the E. H. Sargent Company and was assayed by the U. S. P. XI process. The results (see Table I) are expressed in per cent and also as Gm. of cantharidin representing 10 Gm. of dried drug.

The highest value found for the cantharidin content was 0.654%; the lowest was 0.384%; the mean was 0.554%. In only three cases was the amount above 0.6%, which is the minimum requirement as stated in the United States Pharmacopœia.

The reports on the cantharidin content of Russian Cantharides seem to vary considerably with different workers. There also seems to be a variance from one year to another. Patch's report (5) in 1913 listed the per cent of cantharidin in five samples of cantharides as 0.4, 0.43, 0.4, 0.44, 0.34. The report for the same year by Ewe (6) for twenty-one samples showed nine below the standard of 0.6%.

Table I.—Assay of Cantharides, U. S. P. XI Method

Sample	Gm.	Per Cent	Per Cent Deviation from Mean[a]
1	0.0572	0.572	+ 2.88
2	0.0512	0.512	− 7.58
3	0.0503	0.503	− 9.20
4	0.0654	0.654	+18.05
5	0.0637	0.637	+14.98
6	0.0571	0.571	+ 2.88
7	0.0553	0.553	− 0.18
8	0.0518	0.518	− 6.48
9	0.0593	0.593	+ 7.03
10	0.0593	0.593	+ 7.03
11	0.0596	0.596	+ 7.76
12	0.0568	0.568	+ 2.52
13	0.0484	0.484	−12.63
14	0.0633	0.633	+14.25
15	0.0582	0.582	+ 5.05
16	0.0466	0.466	−18.84
17	0.0566	0.566	+ 2.16
18	0.0572	0.572	+ 2.88
19	0.0384	0.384	−30.66
20	0.0459	0.459	−17.14
21	0.0406	0.406	−26.71
22	0.0408	0.408	−20.93
23	0.0455	0.455	−17.87
24	0.0458	0.458	−17.32

[a] Mean = 0.554%.

By Titration: Procedure: 15-Gm. samples of Cantharides were used. The extraction was carried out according to the directions of the U. S. P. XI process, with the notable exception that the evaporation of the solvent from the extract was performed in a six-inch evaporating dish instead of a tared beaker as directed by the U. S. P. The impure cantharidin obtained after the purification procedure was then dissolved in 25 cc. of acetone, and 25 cc. of $N/20$ alcoholic KOH added, followed by 25 cc. of distilled water. This was evaporated to a volume of about 10 cc., or until acetone and alcohol were no longer detectable. The excess alkali was then titrated with $N/20$ HCl, using phenolphthalein. A blank was run, using 25 cc. of acetone.

One cubic centimeter $N/20$ KOH = 0.0049 Gm. cantharidin. The number of grams of cantharidin found represents 10 Gm. of Cantharides.

Table II.—Cantharidin by Titration

Sample	Per Cent of Cantharidin	Per Cent Deviation from Mean[a]
1	0.666	− 1.62
2	0.652	− 3.69
3	0.666	− 1.62
4	0.701	+ 3.54
5	0.794	+17.28
6	0.701	+ 3.54
7	0.607	−10.33
8	0.627	− 7.38

[a] Mean = 0.677%.

The lowest value found was 0.607% of cantharidin; the highest was 0.794%; the mean for the series was 0.677%. The maximum deviation from the

[1] Note: Severe "cantharidin burns" around the face were experienced while evaporating an alcoholic solution of cantharidin.

mean was 17.28%. All results were above the minimum requirement of 0.6%, U. S. P. The large per cent of deviation in several instances requires further study.

Comparison of U. S. P. XI and Titration Processes.—Samples of Cantharides were assayed by the U. S. P. method. Each residue of cantharidin obtained from these assays was then subjected to titration as previously described. The results of each determination are listed.

Table III.—Comparison of Assay Results

Sample	Per Cent of Cantharidin by U. S. P. XI Method	Per Cent of Cantharidin by Titration Method
1	0.654	0.406
2	0.637	0.347
3	0.572	0.382
4	0.384	0.265
5	0.459	0.436
6	0.406	0.338
7	0.408	0.372
8	0.455	0.396
9	0.458	0.396

Each sample of cantharidin obtained from the assay of Cantharides by the U. S. P. process gave, on titration, smaller values. This indicates that the residue obtained in the U. S. P. process, the weight of which is directed to be called the amount of cantharidin in the drug, is not all cantharidin. This is also evident by mere inspection of the product. It is apparent that the petroleum ether and alcohol mixture, previously saturated with cantharidin, does not remove all of the inert material.

Investigation of the Volatility of Cantharidin.—Cantharidin was dissolved in 100 cc. of a mixture consisting of 1 volume of petroleum ether and 2 volumes of benzene. The solution was then evaporated in a tared 50-cc. beaker on a steam-bath. The time for complete evaporation of the entire solution was noted. After drying at 40° for 20 minutes, the beaker and its contents were weighed and the loss in weight sustained during the process of evaporation found.

Table IV.—Volatility of Cantharidin from Ether-Benzene

Gm. Cantharidin	Time Required for Complete Evaporation	Loss in Weight in mg.	Per Cent Loss in Weight
(*a*) 0.2220	2¼ hours	9.6	4.32
(*b*) 0.0797	1½ hours	2.0	2.50
(*c*) 0.2683	1¾ hours	4.4	1.63
(*d*) 0.2246	1½ hours	2.0	0.80

A similar solution of cantharidin was evaporated in a 6-inch evaporating dish, and, after complete removal of solvent, the cantharidin was transferred to a tared beaker using warm chloroform for this purpose. The chloroform was evaporated quickly and the cantharidin dried and weighed.

Table V.—Volatility of Cantharidin from Ether-Benzene, then Chloroform

Gm. Cantharidin	Time required for Complete Evaporation	Loss in Weight in mg.	Per Cent Loss in Weight
(*a*) 0.1556	8 minutes	0.1	0.06
(*b*) 0.1901	6 minutes	0.2	0.10

In the U. S. P. assay process extraction is carried out with a mixture of petroleum ether and benzene, and this solvent is then evaporated. In carrying out this procedure a small tared beaker is ordinarily used, as the cantharidin is directed to be weighed after purification. The time required for this operation, employing a steam-bath, is about two hours. In the above experiment, using the same solvent and evaporating in a 50-cc. beaker, there was a definite loss of cantharidin. In the second part of the experiment, where evaporation extended over minutes only, the loss was negligible. In the actual assay process by the U. S. P. method, there should be even greater loss of cantharidin, as the presence of extracted fat and other matter should increase the boiling point of the solvent. In the titration method, evaporation is carried out in a six-inch evaporating dish and this process usually does not require more than ten minutes. This helps to explain the higher values obtained by the titration process and its advantage over the U. S. P. process.

Assay of Cantharides.—Using Chloroform for Extraction: As chloroform is an excellent solvent for cantharidin, Cantharides was extracted with this solvent and the titration assay applied. The results from these assays would then serve as an index to the thoroughness of extraction by other solvents, especially the one used in the official process. The extraction procedure was carried out according to the U. S. P. XI directions, using a solvent of 150 cc. of chloroform and 2 cc. of hydrochloric acid. The chloroform was distilled from the extract, the impure residue purified as in the U. S. P., and the final residue of cantharidin then titrated by the method previously outlined.

Table VI.—Assay of Cantharides Using Chloroform Extraction

Sample	Per Cent Cantharidin Found	Per Cent Deviation from Mean[a]
1	0.652	+ 4.48
2	0.588	− 5.76
3	0.627	+ 0.48
4	0.681	+ 9.13
5	0.676	+ 8.33
6	0.519	−16.82

[a] Mean = 0.624%.

The lowest value found was 0.519%; the highest was 0.681%; the mean was 0.624%. The mean of this series is somewhat lower than that found in the previous series in which extraction was made with the official solvent, benzene and petroleum ether. These results indicate that extraction is complete with either solvent, but that the differences may be due to some other factor.

Using Petroleum Ether to Remove Fat: In the U. S. P. method, a mixture of petroleum ether and dehydrated alcohol, previously saturated with cantharidin, is employed to purify the extracted cantharidin. The petroleum ether serves to remove fat and coloring material, while the alcohol removes resin-like material.

Fifteen-gram samples of Cantharides were extracted as previously described following the U. S. P. procedure, using a mixture of two volumes of benzene and one volume of petroleum ether with 2 cc. of hydrochloric acid added. The residue, obtained after filtering off 100 cc. of the extract and evaporating, was washed with small portions of petroleum ether to remove fat and coloring material. The impure cantharidin was then titrated by the method previously described.

Table VII.—Assay of Cantharides after Defatting

Sample	Per Cent Cantharidin Found	Per Cent Deviation from Mean[a]
1	0.593	−0.33
2	0.559	−6.05
3	0.632	+6.05
4	0.588	−1.17
5	0.583	−2.01
6	0.617	+3.69

[a] Mean = 0.595%.

For the per cent of cantharidin, the lowest value found was 0.583%; the highest was 0.632%; the mean per cent was 0.595%. Four of the results were below the U. S. P. requirement of 0.6%.

In the titration method of assay, the use of petroleum ether to remove fat and coloring matter is sufficient; the use of alcohol is entirely unnecessary. As some of the results were below the minimum U. S. P. requirement, it is evident that there must be a loss of cantharidin during the process of removing fat, especially since cantharidin is soluble in fats and oils.

Loss of Cantharidin Due to Presence of Fat.—In order to determine the extent of the loss of cantharidin due to the presence of fat and oil, the following experiments were carried out:

Weighed quantities of cantharidin were dissolved in chloroform and 5 cc. of castor oil were added. The solutions were then placed in a warm place until the chloroform was completely evaporated. The oil was then removed by washing with 10-cc. portions of a solution consisting of equal volumes of petroleum ether and dehydrated alcohol, previously saturated with cantharidin. To prevent washing away of cantharidin, the washings were filtered through cotton, and the retained cantharidin was dissolved out with warm chloroform and added to the remaining crystals. The chloroform was evaporated and the cantharidin dried at 40° for thirty minutes. It was then weighed and the amount of cantharidin lost was found.

Table VIII.—Loss of Cantharidin Due to Presence of Fat

Sample	Gm. of Cantharidin Treated	Mg. of Cantharidin Lost	Per Cent Lost
1	0.2476	23.0	9.28
2	0.0960	19.6	20.41

The above experiment was repeated using petroleum ether in place of the petroleum ether-alcohol mixture for washing out the fat.

While this experiment was performed with castor oil, which may not have the same characteristics as the fat actually present in Cantharides, it nevertheless illustrates that there is considerable loss of cantharidin during the process of removing fat. This factor helps to cause the low or uneven results in both the U. S. P. and titration processes. Any successful method of assay must therefore take into consideration this factor and prevent such loss.

Table IX.—Loss of Cantharidin after Washing Out Fat

Sample	Gm. of Cantharidin Treated	Mg. of Cantharidin Lost	Per Cent Lost
1	0.2892	20.9	7.22
2	0.0967	13.7	14.16

Attempt to Assay Cantharides with Removal of Fat from Alkaline Solution.—If it were possible to convert the cantharidin present in the extract into an alkali salt of cantharidic acid, the fat and other impurities could be removed from this salt by shaking it out in aqueous solution with chloroform, acidifying the fat-free solution and extracting the cantharidin with chloroform. In this way the loss of cantharidin might be prevented. Accordingly, this possibility was investigated, but the troublesome emulsions encountered in attempting to shake out the alkaline solution with chloroform made this method impracticable.

Assay of Cantharides Using Alcohol for Extraction.—As alcohol is not a good solvent for fat, this was used to extract Cantharides and the assay applied. It was recognized that alcohol is also a poor solvent for cantharidin (1:11,500) but, supposedly, enough solvent was used in the process to completely extract it. Furthermore, the presence of hydrochloric acid in the menstruum would be expected to increase the solvent power for cantharidin.

Table X.—Assay of Cantharides Using Alcohol for Extraction

Sample	Per Cent Cantharidin Found
1	0.299
2	0.309

While the process was easily carried out, the results of the assay show that extraction of cantharidin with alcohol is very incomplete, making further work with this solvent useless.

Assay of Tincture of Cantharides.—Procedure: 100 cc. of tincture prepared by the U. S. P. XI process were made alkaline with a 20% solution of potassium hydroxide and heated on a steam-bath until the alcohol was removed. The remaining portion was cooled and then filtered through cotton into a separatory funnel. It was then shaken out with 10-cc. portions of chloroform until the washings were colorless. The aqueous solution was made acid to congo red with hydrochloric acid and extracted with 10-cc. portions of chloroform until all cantharidin was dissolved out. The chloroform was

evaporated and the residue dried. The impure cantharidin was washed twice with petroleum ether (5 cc.) to remove possible traces of fatty acids. By the previously outlined method, the cantharidin residue was titrated and the amount determined.

Table XI.—Results Obtained in the Assay of Tincture of Cantharides

Sample	Gm. of Cantharidin in 100 Cc. of Tincture	Per Cent Deviation from Mean*
1	0.0236	− 4.83
2	0.0265	+ 6.85
3	0.0274	+10.48
4	0.0245	− 1.22
5	0.0221	−10.88

* Mean = 0.0248 Gm.

SUMMARY AND CONCLUSIONS

1. A number of samples of Cantharides have been assayed according to the U. S. P. XI method. It was found that most of these were below the U. S. P. standard of 0.6%. These low results were shown to be due partially to losses of cantharidin, due to its volatility, during the evaporation procedure of the assay, and partially to the method used for the removal of fat from the extracted cantharidin. It was shown that the residue, obtained in the process and weighed as cantharidin, is not all cantharidin, but contains much inert material that is difficult to separate.

2. An improvement on the U. S. P. method has been suggested and tried out. This method requires only a very short period of time for evaporation, as compared to the official method, and determines the cantharidin content of the extracted residue by titration. Although this method yields results higher than the U. S. P. requirement, it has the advantages of being less tedious and time-consuming, and overcomes the loss of cantharidin due to volatilization. It does not overcome the loss of cantharidin sustained in the removal of fat from the extracted residue.

3. In a study of the different parts of the U. S. P. method it has been shown that the solvent of benzene and petroleum ether is satisfactory for the complete extraction of cantharidin. Chloroform was also shown to extract it completely; alcohol was shown to be a poor, unsatisfactory solvent.

4. Attempts to improve the manner in which fats and inert material are removed from the extracted residue were unsuccessful. This part of the assay procedure is in need of further work.

5. Preliminary results on the application of the titration method for estimating the cantharidin content of the tincture are reported. It is recommended that this method is worthy of further investigation for the assay of both the drug and the tincture.

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The Separation and Determination of Aminopyrine in Mixtures

*By L. E. Warren**

INTRODUCTION

The use of synthetic organic substances in medicine is rapidly increasing. During the past two or three decades, the tendency to mix two or more of the synthetics in a single dose has been in evidence. Such mixtures as acetophenetidin, acetylsalicylic acid and caffeine, acetophenetidin and salol, aminopyrine and phenobarbital, aminopyrine and caffeine, and acetylsalicylic acid and phenobarbital are not uncommon. Occasionally, as many as four active medicinal ingredients are found in a single dosage form (acetophenetidin, aminopyrine, caffeine and barbital or phenobarbital).

Satisfactory methods are not available for separating aminopyrine from the mixtures in which it is found. Due to the numerous inquiries which have been received by the Food and Drug Administration, an attempt

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was made to devise methods for the separation of aminopyrine from caffeine, acetophenetidin, barbital, phenobarbital, antipyrine and cinchophen, as well as various combinations of these drugs.

HISTORICAL

Several quantitative procedures for separating aminopyrine from other drugs have been recorded. Patein (1) separated aminopyrine from antipyrine by converting the antipyrine into the insoluble diantipyrine methane, and recovered the aminopyrine (from the filtrate) by extracting with chloroform. Astruc and Pégurier (2) determined aminopyrine by precipitation of the picrate and titrating the excess picric acid. Oliveri-Mandalà and Calderaro (3) determined aminopyrine in the presence of acetylsalicylic acid and antipyrine by heating the mixture with concentrated sodium hydroxide solution in an atmosphere of hydrogen and estimating the liberated amine. Borloz (4) has reviewed and evaluated the foregoing methods for separating aminopyrine from antipyrine.

Machtou (5) determined aminopyrine by precipitation with an excess of mercuric chloride and determination of the excess mercuric salt by the Denigés potassium cyanide–silver nitrate procedure. Miko (6) attempted to separate aminopyrine from acetophenetidin and caffeine by the differences in the solubilities of the three substances in water and ether. Schulek and Menyhárth (7) determined aminopyrine in presence of antipyrine, acetanilid, acetophenetidin and caffeine by treating with 0.1*N* potassium permanganate in presence of 0.1*N* sodium hydroxide and potassium iodide. Sulfuric acid was added and the liberated iodine titrated with sodium thiosulfate. Warren (8) separated aminopyrine from phenobarbital by extracting the aminopyrine from an alkaline solution by chloroform in an automatic extractor. The solution was then made acid and the phenobarbital removed by a fresh portion of chloroform.

Van Giffen (9) separated acetophenetidin, aminopyrine, caffeine, quinine sulfate and magnesium oxide by extracting the acetophenetidin, aminopyrine, caffeine and traces of quinine sulfate with chloroform. Hot diluted sulfuric acid removed the aminopyrine and quinine sulfate from the chloroform and the quinine in this fraction was separated from the aminopyrine by its insolubility in sodium hydroxide solution. Aminopyrine was recovered from the alkaline filtrate by shaking with chloroform. Acetophenetidin and caffeine were separated by the difference in their solubilities in water and by treatment with iodine solution to precipitate caffeine.

By means of complex phosphotungstomolybdic and arsenotungstomolybdic reagents, Wachsmuth (10) colorimetrically determined aminopyrine in the presence of acetanilid, acetophenetidin, antipyrine, caffeine and quinine. Duquénois (11) reported that a solution of cadmium iodide (or potassium cadmium iodide, Marmé's reagent) gives a white precipitate with aqueous solutions of aminopyrine.

Payne (12) separated aminopyrine from phenobarbital by solution of the phenobarbital in *N* sodium hydroxide and removal of the aminopyrine by shaking with chloroform. The alkaline, aqueous solution was acidified and the phenobarbital removed by a mixture of chloroform and ether. He also tried separating the aminopyrine by combining it with sulfuric acid (1 + 9) and removing the phenobarbital with an immiscible solvent. Sinton and Rotondaro (13) separated aminopyrine from caffeine and antipyrine by shaking the mixture with chloroform in presence of 5 per cent sulfuric acid.

EXPERIMENTAL

Tests were carried out to ascertain whether or not the aminopyrine cadmium iodide complex described by Duquénois (11) was sufficiently insoluble to be of service for a quantitative determination. Samples of this complex were prepared from aminopyrine and cadmium iodide. The solubility of this material at 8–10° was found to be about 1 part in 1000 parts of water. This value was thought to be too great for an accurate quantitative procedure.

Due to some encouraging results (unpublished) for the separation of mixtures of acetylsalicylic and salol, acetophenetidin and salol, and acetanilid and salol by methods based upon their differential solubilities in organic solvents, an attempt was made

to separate aminopyrine from various mixtures by such a procedure. According to figures given in Table I, it seems likely that aminopyrine and cinchophen could be separated in this manner by the difference in their solubilities in carbon tetrachloride. Some tests were made and the results gave recoveries of 115 per cent for aminopyrine. Possibly the high results may be explained by some form of combination between aminopyrine and cinchophen, the resulting product having a different solubility in carbon tetrachloride. Since most of the mixtures under consideration in this paper contained aminopyrine and some acidic medicinal, it was thought that similar difficulties might be encountered throughout and this method was abandoned.

After numerous trials, the following method was adopted:

From 1 to 2 Gm. of the mixture were weighed into a separator, 25 cc. of approximately *N* sulfuric acid added and the mixture shaken with 40 cc. of chloroform (certain excipients do not dissolve). The extraction was repeated five times using 25-cc. portions of chloroform. Each chloroform fraction was washed successively through 10 cc. of 3.5 per cent sulfuric acid and 10 cc. of 1 per cent hydrochloric acid. The chloroform was filtered through cotton into a weighed Erlenmeyer flask. Most of the chloroform was recovered and the remainder allowed to evaporate spontaneously while the container was rotated in an inclined position. A few cc. of anhydrous ether were added and the residue again heated to dryness to remove last traces of chloroform (14). The residue was dried at 80° and weighed as anhydrous caffeine.

Table I.—Solubilities of Seven Organic Substances in Seven Solvents

(Compiled from various sources)

	(1 Gm. of Substance Soluble in Stated Number Cc. of Solvent)						
Substance	Alcohol	Benzene	Carbon Tetrachloride	Ether	Chloroform	Petroleum Benzin	Water
Acetophenetidin	15	1755	1715	130	14	10,200	1310
Aminopyrine	1.5	12	7.15	13	1	238	18
Antipyrine	1.3	14	61	43	1	3220	Less than 1
Barbital	14	2000	9100	35	75	100,000	130
Caffeine (hydrated)	66	100	700	530	5.5	20,200 17,600	46
Cinchophen	120	4771 5380	17,500	100	400	60,000	11,000
Phenobarbital	8	700	Slightly soluble	13	40	18,300	1000

Separation of Aminopyrine from Caffeine.—Preparations of aminopyrine and caffeine only do not appear to be on the market, but mixtures of these ingredients with others, such as acetophenetidin, extract of hyoscyamus and phenobarbital, are available. Since the analysis of the more complex preparations involves the separation of the aminopyrine from the caffeine, it was considered important that methods for the separation of these two substances be studied first.

In the preliminary quantitative tests, it was noted that *N* hydrochloric acid[1] did not completely hold the aminopyrine when the mixture was shaken with chloroform to remove the caffeine. After some experimentation and correspondence with other members of the Administration, sulfuric acid (1 + 9) was chosen for further study. Most of the results obtained in the earlier portion of this study were obtained with this strength of acid and are so designated in the tables. Later experiments indicated that the optimum strength of sulfuric acid was 5 per cent for the extractions and 3.7 per cent for the first washing as recommended by Sinton and Rotondaro (13). In the later trials, the author employed one further washing of the chloroform extract with one per cent hydrochloric acid.

The washings were added to the mixture in the first separator, the solution made slightly alkaline with ammonia T.S. and shaken out with 25-cc. portions of chloroform until all of the aminopyrine had been removed. Each chloroform fraction was washed through 10 cc. of water containing a few drops of ammonia T.S., the chloroform filtered through cotton and the filtrate collected in a weighed Erlenmeyer flask. The solution was evaporated and the residue treated as for caffeine in the preceding paragraph.

Known mixtures of aminopyrine and anhydrous caffeine were prepared with and without starch as a diluent. Portions of these mixtures were analyzed according to the method outlined above and the results are summarized in Table II.

Determination of Aminopyrine and Acetophenetidin in Mixtures.—Five analyses were made by the above method on an authentic mixture containing 18.43 per cent acetophenetidin and 64.0 per cent aminopyrine. The recoveries for acetophenetidin ranged from 98.4 to 101.1 per cent with an average of 100 per cent and the aminopyrine determinations ranged from 99.1 to 101.1 per cent with an average of 100.2 per cent.

Separation of Aminopyrine and Barbital.—A mixture of aminopyrine, barbital and starch in approxi-

[1] Hydrochloric acid of this concentration was selected because this is employed as a solvent in the A. O. A. C. official method for the assay of aminopyrine tablets. However, in this case the acid is neutralized before removal of the aminopyrine by chloroform.

Table II.—Analyses of Aminopyrine and Caffeine Mixtures

Number of Analyses	Acid Used	Aminopyrine			Caffeine		
		Found, %	Theory, %	Recovery, %	Found, %	Theory, %	Recovery, %
Without diluent							
3	*N* HCl	89.5	90.0	99.4	10.58	10.03	105.5
2	H_2SO_4 (1 + 9)	91.13	89.7	101.6	10.11	10.28	98.3
5	H_2SO_4 5%	87.93	88.11	99.8	12.19	11.89	102.5
With added starch							
6	H_2SO_4 5%	70.33	71.59	98.2	9.83	9.57	102.7

mately the proportions of commercial preparations was subjected to analysis by the following methods:

1. The method previously outlined for the separation of aminopyrine and caffeine was modified slightly due to the low solubility of barbital in chloroform. A mixture of chloroform and ether (2 + 1) was used and a greater number of extractions were required to completely remove the barbital.

2. The material was suspended in water, the mixture made alkaline with sodium hydroxide T.S. (to hold the barbital) and the aminopyrine extracted with small successive portions of chloroform. The chloroformic solutions were united, washed successively with water containing 1 per cent of sodium hydroxide and 1 per cent of ammonium hydroxide and evaporated. The residue was treated as previously described and weighed as aminopyrine. The alkaline solution from which the aminopyrine had been removed was then acidified with sulfuric acid T.S. and the barbital removed by shaking with chloroform. The chloroform solution was washed with water, the chloroform evaporated, the residue treated with the usual precautions and weighed as barbital.

In five analyses by the first method, the recoveries of barbital ranged from 100.1 to 101.4 per cent with an average of 100.7 per cent. The recoveries of aminopyrine ranged from 99.3 to 100.8 per cent with an average of 99.6 per cent. In four analyses by the second method, the barbital recovered ranged from 97.9 to 101.8 per cent with an average of 100.3 per cent and the aminopyrine recoveries from final assays ranged from 98.8 to 100.7 per cent with an average of 99.5 per cent.

Separation of Aminopyrine and Phenobarbital.—Mixtures of aminopyrine and phenobarbital with and without starch were prepared. These were assayed by each of the two methods previously described for barbital mixtures, except that two of the trials were made with *N* hydrochloric acid.

The recoveries for phenobarbital by the first method from two trials using *N* hydrochloric acid were 109.0 per cent and 106.0 per cent. The recoveries for aminopyrine in the same trials were 99.8 and 98.8 per cent. The recoveries for phenobarbital from seven assays in 5 per cent sulfuric acid medium ranged from 99.8 per cent to 103.8 per cent; average 101.6 per cent. Recoveries for aminopyrine in the same tests ranged from 99.0 to 100.0 per cent; average 99.7 per cent.

The recoveries in five assays for aminopyrine, using the second method, ranged from 99.5 to 100.2 per cent; average 99.8 per cent. Recoveries of phenobarbital in the same tests ranged from 99.2 to 107.6 per cent; average 101.5 per cent.

Separation of Aminopyrine, Antipyrine and Caffeine.—A standard mixture of aminopyrine, antipyrine, anhydrous caffeine and starch was prepared and the preparation was assayed by the method previously described for the separation of aminopyrine from caffeine. The method was applied to the separation of the aminopyrine from the total antipyrine and caffeine, but no attempt was made to separate the antipyrine and caffeine since the Association of Official Agricultural Chemists (15) has already adopted a method for this purpose.

In six experiments the recoveries for total antipyrine and caffeine ranged from 100.0 to 101.3 per cent; average 100.7 per cent. For aminopyrine the recoveries ranged from 98.9 per cent to 102.3 per cent; average 100.2 per cent.

Separation of Aminopyrine and Cinchophen.—Theoretically, the method used for the separation of aminopyrine from caffeine should be applicable to the separation of aminopyrine and cinchophen. However, owing to the relatively low solubility of cinchophen in chloroform (1 Gm. in 400 cc.), it was found expedient to use a mixture of chloroform and ether (2 + 1) instead of chloroform for its extraction. Two methods were tried as in the assay of barbital.

In four assays made according to the first method on a sample containing 40.63 per cent cinchophen and 18.87 per cent aminopyrine, the recoveries for cinchophen ranged from 99.6 to 100.5 per cent with an average of 100 per cent and those for aminopyrine ranged from 98.5 to 101.9 per cent with an average of 99.6 per cent.

Five assays made by the second procedure on the same sample gave recoveries of cinchophen ranging from 99.1 to 103.0 per cent with an average of 100.7 per cent, and those for aminopyrine ranging from 99.7 to 104.3 per cent with an average of 101.7 per cent.

Separation of Aminopyrine, Acetophenetidin, Caffeine and Phenobarbital in Mixtures.—The results from the separation of aminopyrine from caffeine, acetophenetidin, barbital, cinchophen and phenobarbital having been reasonably successful, it was decided to try the separation of four medicinal in-

Table III.—Analyses of Aminopyrine, Phenobarbital, Caffeine and Acetophenetidin Mixtures

	Aminopyrine			Phenobarbital			Caffeine			Acetophenetidin		
Sample	Found, %	Theory, %	Recovery, %	Found, %	Theory, %	Recovery, %	Found, %	Theory, %	Recovery %	Found, %	Theory, %	Recovery, %
1	47.18	47.23	99.9	2.00	2.03	98.5	4.24	4.06	104.4	23.87	24.34	98.1
2	47.27	47.23	100.1	1.95	2.03	96.1	4.12	4.06	101.5	24.05	24.34	98.8
3	46.92	47.23	99.3	1.98	2.03	97.5	4.11	4.06	101.2	24.33	24.34	100.0
4	45.15	45.08	100.2	2.13	2.13	100.0	5.51	5.19	106.0	24.14	24.78	97.4
5	45.01	45.08	99.8	2.19	2.13	102.8	5.21	5.19	100.4	25.58	24.78	103.2
6	45.20	45.08	100.3	2.09	2.13	98.1	5.13	5.19	98.8	25.19	24.78	101.7
7	45.24	45.08	100.4	2.16	2.13	101.4	5.09	5.19	98.1	25.41	24.78	102.5
8	43.74	45.08	97.0	2.06	2.13	96.7	5.18	5.19	99.8	25.47	24.78	102.8
9	45.93	45.08	101.9	2.11	2.13	99.1	5.08	5.19	97.9	23.32	24.78	94.1
10	45.29	45.08	100.5	2.23	2.13	104.7	5.21	5.19	100.4	24.71	24.78	99.7
11	44.15	45.08	97.9	2.11	2.13	99.1	5.25	5.19	101.2	24.56	24.78	99.1
Average	45.55	45.67	99.7	2.09	2.10	99.5	4.92	4.88	100.8	24.60	24.66	99.8

gredients when mixed. Accordingly, a mixture containing aminopyrine, caffeine, acetophenetidin and phenobarbital with starch[2] was prepared. This was subjected to the following method:

Aminopyrine.—About 5 Gm. of the powdered tablet mixture was weighed into a separator, 25 cc. of *N* sulfuric acid and 75 cc. of chloroform added and the mixture well shaken. The chloroform was drawn off and washed successively with 10 cc. of 3.5 per cent sulfuric acid and 10 cc. of 1 per cent hydrochloric acid. The chloroform was filtered into a weighed Erlenmeyer flask, the mixture in the first separator extracted with six more portions of 50 cc. each of chloroform, each portion washed successively through the acidified waters as before and collected in the weighed flask. This chloroform solution was retained for the recovery of acetophenetidin, caffeine and phenobarbital. The acid washings in the second and third separators were added to the first separator, the mixture made alkaline with ammonia T.S. and the aminopyrine removed by successive extractions with 25-cc. portions of chloroform. The chloroform extracts were washed in a second separator with 5 cc. of water containing a few drops of ammonia T.S. and the solvent filtered through cotton into a tared flask. Most of the solvent was recovered and the remainder evaporated while the container was rotated in an inclined position. A few cc. of anhydrous ether were added and the mixture again evaporated. The residue was dried at 80° and weighed as aminopyrine.

Phenobarbital.—The chloroformic solution of acetophenetidin, etc., was evaporated to 50 cc. and shaken 5 times with 25-cc. portions of 0.1*N* sodium hydroxide which removed phenobarbital as the soluble sodium compound. The aqueous alkaline solutions were drawn off and each washed with 5 cc. of chloroform to remove the last traces of acetophenetidin and caffeine. The chloroformic washings were added to the original chloroformic solution of caffeine and acetophenetidin. The aqueous, alkaline solution was acidified and shaken with chloroform-ether (2 + 1) to remove the phenobarbital.[3] The solvent was washed, evaporated and the residue of phenobarbital dried and weighed in the usual manner.

Acetophenetidin and Caffeine.—Acetophenetidin and caffeine were separated according to method described by Grove (16).

The results of the analysis of two prepared samples by the above technique are given in Table III.

[2] Tablets of this approximate composition are on the market.

[3] An automatic extractor may be used to advantage for this operation.

COMMENTS

Of the various methods of extraction tried, the most satisfactory was the removal of all of the drugs except aminopyrine by shaking with chloroform or chloroform-ether mixture in the presence of 5 per cent sulfuric acid. The aminopyrine remained in the acid solution and was removed by chloroform after the addition of alkali.

Traces of aminopyrine were persistently carried over in the chloroform extractions from acid solutions. This is shown by the generally high results obtained for acetophenetidin, barbital, caffeine, phenobarbital, etc., and by the faintly yellowish color of these residues when heated for some time at 100°. The presence of aminopyrine in these fractions usually may be shown by heating the residues of caffeine, phenobarbital, etc., with water, cooling, filtering and adding a few drops of cadmium iodide solution to the filtrate. In most cases, however, the error due to the extraction of aminopyrine from acid solutions was negligible.

SUMMARY

Two quantitative methods are described in this paper but both are not applicable to all separations involving aminopyrine. Methods have been developed for the determination of aminopyrine, acetophenetidin, antipyrine, barbital, caffeine, cincho-

phen and phenobarbital in mixtures and by a combination of these methods with A. O. A. C. procedures, determinations of as many as four of the constituents in the same mixture have been made. The recoveries by the recommended methods range from about 98 per cent to about 104 per cent.

Precipitation of the aminopyrine as the double salt with cadmium iodide or with mercuric chloride was not entirely satisfactory. Attempts to separate the several substances by the differences in their solubilities in various solvents were not satisfactory.

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NOTICE

Send in papers for Richmond meeting to Section Secretaries by March 15th.

The Determination of Camphor in Camphor Liniment

An Accurate and Simplified Volatilization Method

*By Solomon M. Berman**

The last three revisions of the U. S. Pharmacopœia have presented as many different assay methods for Liniment of Camphor. The U. S. P. IX method involved removal of the camphor from the oily vehicle by means of hot alcohol vapor, the camphor in the distillate being measured polariscopically. The U. S. P. X assay was essentially that proposed by Miller (1) who stated that 90 minutes heating at 110° in a platinum dish was sufficient to volatilize the camphor, without decomposing the oil. He criticized the earlier method as impractical. Previously, Cook (2) had suggested a 3-hour heating at 100° for removing 97.5% of the camphor in a 20% liniment, with a suitable correction factor. Lothian (3) recommended the use of petri dishes and heating on the water-bath for one hour. He noted that the residue gained in weight on further heating, while the oil, used in preparing the liniment, did not gain. He concluded that blanks were not dependable.

In a series of papers, Poe and others (4) made a detailed report of studies on the assay of Camphor Liniment. They found that the U. S. P. X method gave low results on the official liniment as well as on liniments prepared from the other common fixed vegetable oils. They also observed that aluminum dishes gave the lowest results in any series of determinations, while glass dishes were relatively satisfactory and tin or lead vessels were best. These workers found that the most serious error in the volatilization procedure was due to oxidation of the oil at the temperature used, the effect increasing with time and more pronounced when aluminum vessels were employed. Subsequent studies of heating *in vacuo*, in nitrogen, in carbon dioxide, and in the presence of anti-oxidants, led to their

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recommending the use of the vacuum oven at 115–120° for two hours, as yielding results nearest to theoretical. Their data largely support this recommendation, but also show decomposition of oil under those conditions amounting to 0.12%, which corresponds to 0.48% of the camphor in a 20% liniment.

The method at present official in the U. S. P. XI is also official (with slightly different wording) in the N. F. VI for the assay of Ampuls of Camphor, and is due to Grantham and his collaborators (5). Their results are in essential accord with those of Poe (4) for the parallel method, and for eleven determinations ranged from 98.2% to 102.5% recovery, with an average of 99.9%. It should be noted that the collaborating analysts reported from three to six hours as the necessary heating period.

Overbye and Schoetzow (6), using various 20% liniments, found by this method amounts of camphor varying from 18.63% to 19.89%, after correcting for loss in drying the oils alone. The range of results corresponds to recovery of from 93.15% to 99.45% of the camphor content. They concluded that a blank was needed on the particular oil used in preparing the liniment.

A method was sought which would be rapid and accurate, and which would not cause any significant change in the oily vehicle of the Camphor Liniment. The present report is believed to describe such a method.

EXPERIMENTAL

Three batches of Camphor Liniment were prepared, corresponding to the upper and lower limits as well as to the nominal 20% formula requirement of the U. S. P. XI (range—19% to 21% camphor). The camphor used was evidently a synthetic product in view of its small optical activity. (Five grams in 50 cc. alcohol showed 3.8° in 100-mm. tube.) The cottonseed oil was a commercial edible oil, meeting the U. S. P. requirements, and about two year old.

The U. S. P. method of preparation was followed in each case, the weighed camphor (by difference) being added to the warm cottonseed oil, contained in an Erlenmeyer flask; the flask tightly stoppered with a foil-lined cork and agitated on a rotating machine for several hours until a perfectly clear solution was obtained. Liniment *A* consisted of 81.0072 Gm. of oil and 19.5761 Gm. of camphor, corresponding to 19.463% of camphor. Liniment *B* consisted of 79.9993 Gm. of oil and 20.1240 Gm. of camphor or 20.099% of camphor. Liniment *C* consisted of 79.0031 Gm. of oil and 20.9234 Gm. of camphor or 20.939% of camphor.

In the initial experiment, only Liniments *A* and *B* were used. Approximately 5-cc. samples were weighed into petri dishes of 90 mm. diameter, each dish having as tare a similar petri dish, carried along with it through the conditions of the analysis. A 4-hole water-bath was used, so only two liniments and their tares could be run simultaneously. The bath was electrically heated, the water temperature being 99–100°. A draft of the hood window-sash type was not available, a medium size electric fan operating at moderate speed in fixed direction being used instead. The air current was mainly across the dish, but partly into it. A 30-minute heating was followed by 15-minute heatings to constant weight, the weighings being made after each heating. Five to 10 minutes standing in a current of air, preferably on a conducting surface, was sufficient to bring the vessels and contents to room temperature. The results are given in Table I.

Table I.—Recovery of Camphor Based on Loss in Weight of Liniment

Liniment	Sample Wt., Gm.	In Petri Dishes On Water-Bath with Air Draft — Recovered, % — 30 Min.	45 Min.	60 Min.
A	4.5445	99.49	99.85	99.85
A	4.8950	99.42	99.88	99.84
B	4.9458	98.72	99.80	99.86
B	4.5009	99.34	99.83	99.81

In another series, an 8 hole gas-fired water-bath was used, duplicate samples of the 3 liniments being run concurrently, with a single petri dish as tare. A considerable differential existed in the degree of draft afforded to the dishes at opposite ends of the bath. The size of sample was permitted to vary from 3.0 to 5.3 Gm. In no case did the oil completely cover the floor of the dish even when warm. The water-bath temperature was 99°. A maximum thermometer, or other device for measuring the highest temperature attained by the oil samples, was not available. However, a beaker of oil immersed in the steam with frequent agitation showed a maximum of 95° in the 30-minute period. It is estimated that the temperature of the liniment samples varied between 70° and 90° depending upon their nearness to the fan. The data are given in Table II.

Table II.—Recovery of Camphor Based on Loss in Weight of Liniment

Liniment	Sample Wt., Gm.	In Petri Dishes on Water-Bath with Air Draft — Recovered, % — 30 Min.	45 Min.	60 Min.
A	4.6919	97.82	99.42	99.62
B	4.5423	98.59	99.63	99.57
C	5.3062	96.44	99.41	99.68
A	4.0464	98.17	99.24	99.38
B	3.2661	99.55	99.64	99.60
C	3.0040	99.27	99.54	99.56

Aluminum dishes similar in size to the petri dishes, and of rather heavy construction, were tried. They were found objectionable because they were too hot to be handled safely with the fingers on removal from the bath, the use of tongs being inadvisable as they might readily come in contact with the oil. The experiment was abandoned after 45 minutes of heating, 9 determinations showing from 97.67% to 100.22%, before constant weight was attained.

Test was then made of light weight aluminum dishes having a diameter of 57 mm. (against 90 mm. for the petri dishes), a depth of 10 mm., and weighing about 4 Gm. In contrast to the larger aluminum dishes, these were found very convenient. The size of the sample was reduced to about 3 Gm. so as to barely cover the floor of the vessel. In the first two trials reported in Table III, the tare was carried through the conditions of the assay, while in the rest the tare remained on the balance pan. The bath temperature was 100°.

Table III.—Recovery of Camphor Based on Loss in Weight of Liniment

		In Aluminum Dishes on Water-Bath with Air Draft		
		Recovered, %		
Liniment	Sample Wt., Gm.	30 Min.	45 Min.	60 Min.
B	3.0215	99.87	99.86	....
C	2.8546	99.98	99.91	....
A	2.6831	98.98	99.85	99.83
A	2.9176	100.03	100.18	100.13
B	2.6549	99.59	99.94	99.93
B	2.8975	96.61	99.20	99.75
C	3.2998	97.71	99.62	100.02
C	3.1048	99.49	99.77	99.74

A partial study was made of the effect a lower bath temperature would have. An effort was made to equalize the fan effects for this trial.

Table IV.—Recovery of Camphor Based on Loss in Weight of Liniment

		In Petri Dishes on 95° Water-Bath with Air Draft			
		Recovered %			
Liniment	Sample Wt., Gm.	30 Min.	45 Min.	60 Min.	75 Min.
A	5.3092	87.4	94.6	99.13	99.74
A	5.3885	81.6	93.5	98.51	99.69
B	5.2293	95.5	98.9	99.48	99.68
B	4.9084	94.0	97.6	99.18	99.55
C	4.5455	97.0	99.1	99.66	99.76
C	4.6948	97.9	99.5	99.69	99.70

Beakers of 150-cc. capacity and 60-mm. diameter were also tried, immersed in the 95° bath. A 30-minute heating gave 88% to 94% recovery on four samples. Their use indicated no advantage, and was not tested further.

Table V.—Comparative Assays of Commercial Camphor Liniment

	In Petri Dishes on Water-Bath with Air Draft			U. S. P. XI, 2 Hours 110°—CO_2	
	Recovered, %				
Liniment	30 Min.	45 Min.	60 Min.		
(1)	17.14	17.20	17.19	17.14,	17.13[a]
(2)	18.78	18.78	...	18.77,	18.79[a]
(3)	25.13	25.09	...	25.21,	25.22[b]
(3)	24.00	25.02	25.07	...	...
(4)	17.47	17.65	17.60	17.59,	16.58[b]
(5)	16.62	16.84	16.88	16.93,	16.93[b]

[a] Routine analyses by D. W. McLaren.
[b] Routine analyses by R. L. Herd.

The new method was then used to assay five commercial liniments, in comparison with the U. S. P. XI assay conducted at the same time.

In the foregoing experiments necessity dictated the use of an electric fan. When a steam-bath with cross draft became available, a comparison was made of the two types of ventilation involved. The cross draft was produced by a blower creating suction through a slot at the bottom of the baffle forming the back of the hood which contained the steam-bath. The front of the hood was completely open, the resulting draft being more moderate than if a sash had been used, and quite lacking the blowing effect of the fan. Table VI shows a single comparison, using a commercial liniment and petri dishes.

Table VI.—Comparative Assay of Commercial Camphor Liniment

	In Petri Dishes on Steam-Bath	
	Recovered, %	
	30 Min.	45 Min.
Hood draft	19.74	19.75
Electric fan	19.73	19.75

Six samples of cottonseed oil were treated under most rigorous assay conditions, three of the samples receiving collaborative attention. The collaborators were instructed to:

"Weigh accurately from 2 to 5 Gm. of sample in a shallow, flat-bottomed dish of thin-walled construction and such size that the sample barely covers the floor of the dish. Use a similar dish as tare, and carry through the conditions of analysis. Heat the dish on a boiling water- or steam-bath for 30 minutes, in a strong current of air. Cool to room temperature and weigh. Heat as before, for 15 minutes, and again weigh. Repeat this procedure two more times, so that the total heating time is 75 minutes."

Four of the collaborators were directed to use glass dishes, and all chose petri dishes. The two other collaborators used light aluminum dishes (5.7 cm. diameter) to check on any possible difference due to vessels of this material

Table VII.—Maximum Gain in Weight of Cottonseed Oils

	In Petri Dishes[b] on Steam-Bath with Air Draft				
	Gain in Wt., %				
Collab.[a]	Oil No. 1	Oil No. 2	Oil No. 3	Oil No. 4	Oil No. 5
H. R.	0.03	0.02	0.01	....	....
	0.02	0.02	0.02	....	....
C. F. B.	0.02	0.02	0.01	....	....
	0.04	0.04	0.02	....	....
J. D. C.	0.03	0.03	0.05	....	....
	0.02	0.05	0.04	....	....
D. M. T.	0.03	0.01	0.00	....	....
	0.03	0.00	0.03	....	....
	In Aluminum Dishes[c]				
R. L. H.	0.05	0.03	0.06	....	....
	0.03	0.05	0.04	....	....
S. M. B.	0.02	0.02	0.02	0.03	0.01
	0.02	0.00	0.05	0.01	0.01
Average	0.03	0.02	0.03	0.02	0.01

[a] Collaborators: Harry Rogavitz, New York; C. F. Bruening, Baltimore; James D. Curphey, Philadelphia; D. M. Taylor, Philadelphia; R. L. Herd, Buffalo; the author all members U. S. Food and Drug Administration.
[b] Sample weight from 2.99 Gm. to 3.71 Gm.
[c] Sample weight from 2.88 Gm. to 4.46 Gm.

The first four cottonseed oils of Table VII were commercial oils from as many different manufacturers of Camphor Liniment and had been bought for liniment vehicles. One of them was also intended for preparation of Ampuls Camphor in Oil N. F. (The N. F. VI permits use of "any bland fixed oil.") Oil No. 5 was the base of the laboratory-made liniments. A 5.1-Gm. sample of this oil heated for a single 45-minute period gained 0.03% in a petri dish.

The sixth oil was the residue from the commercial Camphor Liniment of Table VI. The residue from the "hood draft" treatment gained 0.07% after two hours' further heating, while the residue from the "fan" treatment gained 0.17% after two and one-half hours' heating preceded by an equal period of exposure at room temperature. The over-all exposure to air of the collaborative samples was four to five hours.

DISCUSSION

So far as the writer has been able to ascertain, the use of efficient ventilation as a means of volatilizing the camphor at oil temperatures below 100° has not previously been suggested. Many laboratories have ovens equipped with fans in constant motion. It is fairly certain that where such was the case the U. S. P. X method gave good results. The foregoing tables show clearly that a high order of accuracy may be attained in the assay of Liniment of Camphor by simply heating the liniment in a current of air on a bath maintained at close to 100°, and weighing the residue at relatively short intervals. A difference between two successive weighings of less than 1 mg. per Gm. sample would be a sufficient assurance that the volatilization of the camphor was as complete as usually necessary. Under well-standardized conditions a single 30-minute heating should be enough to yield better than 99% recovery. In view of the fact that such conditions would not be universally obtainable it is well that no error, and but little inconvenience, is introduced by heating to constant weight.

The insignificance, analytically, of the effect of the assay conditions on the cottonseed oil base is shown in three ways: (*a*) the very slight gain in weight of five different cottonseed oils subjected to assay conditions, both in glass and aluminum, (*b*) the uniformly excellent recovery of camphor from three laboratory made liniments and (*c*) the very slight gain in weight of the residues on continued heating. It must be inferred that the oxidative changes noted by previous workers were associated with the higher temperatures and longer heating periods they employed.

Since it appeared more informative to report camphor recovery than to report per cent camphor in any given liniment, it was necessary to recalculate the data of others for comparison. Obviously, in the case of the commercial samples, where the true camphor content was unknown, only the found per cent camphor in the liniment could be shown.

The tabulated comparison of results by the proposed method and by the U. S. P. XI assay shows that the two procedures can yield results in excellent agreement. From a practical aspect, however, the proposed method presents several important improvements: (*a*) it enables a large number of determinations to be made concurrently; (*b*) it makes unnecessary the monopoly of a relatively expensive and busy piece of equipment, the electric oven, for two hour periods; (*c*) it eliminates the need for accessories, such as drying tubes, delivery tubes and carbon dioxide generator; and (*d*) it furnishes the analyst with an objective record of the completed loss of volatile matter from his sample.

SUMMARY

(1) The literature on volatilization methods for assaying Liniment of Camphor is briefly reviewed.

(2) A simplified method is described, consisting essentially of heating the Liniment of Camphor on a boiling water-bath, in a current of air, to constant weight.

(3) A number of results are tabulated to show the rapidity and dependability of the method under varying conditions. Twenty-four determinations averaged 99.75% recovery.

(4) It is shown that aluminum vessels may be used instead of glass.

(5) The new method is compared with the official assay of the U. S. P. XI (and of the N. F. VI for Ampuls of Camphor) and certain practical advantages are noted.

ACKNOWLEDGMENT

The author wishes to thank his collaborators for their assistance, Mr. A. G. Murray and Dr. F. H. Wiley for review and criticism of the paper, and finally Mr. T. F. Pappe for permission to do the work reported here.

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Determination of Iodine in Thyroid

Comparison of a Rapid Method with the U. S. P. XI Method

By Charles E. Nicklaus and Nelson Tippett**

Previous investigations have emphasized the need of an assay method for Thyroid, that is both quick and accurate. Tippett (1) simplified the U. S. P. XI Method and increased the accuracy by consideration of a titration time factor. The one inherent disadvantage of the alkaline ash method remained however—one assay taking the better part of a working day. Nicklaus has adapted the Matthews, Curtis and Brode modification of the Leipert procedure (2) to Thyroid products and obtained a method having the triple advantage of speed, accuracy and manipulative ease. In substance this method depends upon an initial chromium trioxide oxidation and distillation in a special, but quite simple apparatus, and a subsequent titration with hundredth-normal sodium thiosulfate, the complete procedure requiring a maximum time of one hour. Over a period of six months, numerous assays by each method have shown practically no variation. For the appended table a representative few have been chosen.

* Control Laboratories of The Columbus Pharmacal Co., Columbus, Ohio.

If care is taken to use reagent grade chemicals, no blank is needed.

EXPERIMENTAL

Preparation of Reagents.—30*N* Chromium Trioxide: Dissolve 165.0 Gm. chromium trioxide in 100 cc. water.

N Potassium Carbonate: Dissolve 14.0 Gm. of the salt in 100 cc. water.

0.2*M* Potassium Permanganate: Dissolve 3.16 Gm. in 100 cc. water.

1.5*N* Sodium Nitrite: Dissolve 10.4 Gm. in 100 cc. water.

5*M* Urea Solution: Dissolve 30.0 Gm. in 100 cc. water.

Determination of Iodine.—Weigh accurately about 0.5 Gm. of Thyroid and place it in a 500-cc. Kjeldahl flask (containing an antibump) and wash down the inside of the flask with about 10 cc. of distilled water. If the assay is to be run on a tablet, a representative number are weighed and powdered and an aliquot part of the powder, equivalent to about 0.5 Gm. of Thyroid, is treated in the same manner. No trouble is experienced with tablets of any grainage.

With constant rotation of the flask, slowly add 10 cc. of 30*N* chromium trioxide, and after the reaction has subsided add slowly 50 cc. of sulfuric acid. Place the flask in a ring stand and fit it with a three-hole rubber stopper containing a 0–220°C. thermometer and two tubes for ventilation. Heat the contents at a temperature of 210–220° C. until the liquid acquires a clear bluish green coloration; when the bubbles cease to aggregate on the surface of the liquid, oxidation is complete. Prolonged heating at high temperature results in the formation of an insoluble chromium sulfate precipitate leading to bumping difficulties during distillation. Remove the flask from the ring stand and cool it first under the hot-water tap and then under the cold. Wash the thermometer and remove it. Add 100 cc. of distilled water cautiously while rotating the flask. Connect the flask with the distillation apparatus. To a 250-cc. flask containing an antibump, add 2-cc. of *N* potassium carbonate and place the flask under the condenser stem in such a manner that the tip of the stem dips into the liquid. Place 10 cc. of phosphorous acid 50% in the reservoir on the top of the apparatus and after bringing the liquid to a boil so as to wet the glass joints, allow the acid to run in slowly by turning the stop-cock. Rapidly distil over 75 cc. Lower the flask containing the distillate, open the stop-cock, and wash down the condenser stem permitting the washings to join the distillate. Remove the flask to a burner and boil down to a volume of 4.5 to 5.0 cc. Wash the antibump and remove it. Place the flask on a steam-bath and add from a dropper enough 0.2*M* potassium permanganate to establish a permanent wine-red coloration. Heat five minutes with frequent rotation. Add 10 drops of syrupy phosphoric acid and continue to heat for five minutes. No decoloration of the permanganate

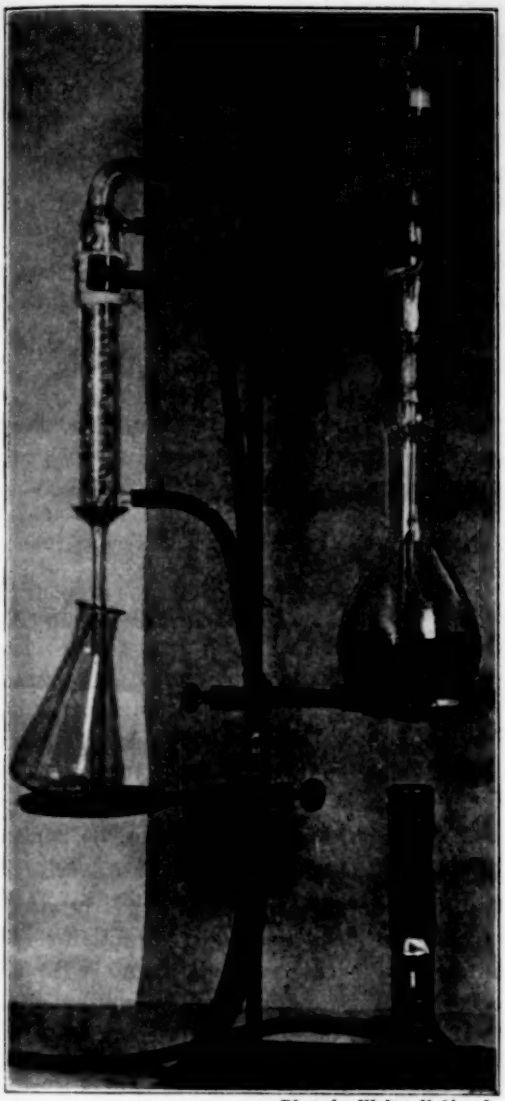

Photo by Walter Hobbs, Jr.

Fig. 1—Photograph of distillation apparatus.

should occur. If decoloration does occur, more permanganate should be immediately added. Then add just enough 1.5*N* sodium nitrite to decolorize it, plus one drop in excess. Heat five minutes. Add eight drops of 5*M* urea solution and after five minutes' heating remove from the steam-bath and cool to room temperature. The flask should be rotated frequently during the five-minute heating intervals, and additions of the permanganate, acid, nitrite and urea solutions should be made dropwise directly into the liquid, care being taken not to get any on the sides of the flask. Add crystalline potassium iodide in slight excess and immediately titrate the liberated iodine with hundredth-normal sodium thiosulfate, using starch T.S. as the indicator. Each cc. of hundredth-normal sodium thiosulfate is equivalent to 0.0002115 Gm. iodine.

Apparatus.—The apparatus designed for the distillation is presented in the accompanying photograph and figure. *A* is the oxidation and distillation flask which has the usual Kjeldahl shape and which may be of any convenient size (500-cc. capacity found to be quite satisfactory). *B* is the entry tube which is 16.5 cm. in length with an inside diameter of 3 mm. and an outside diameter of 9 mm. It should reach to within 5 mm. of the bottom of the flask. *C* is a standard ground glass joint of 25- or 30-mm. size. *D* is a cup into which solutions are measured before they are let through the stop-cock and entry tube into the flask. *G* is a coil condenser which has a dew cup attached. The condenser stem is about 17 cm. long with an inside diameter of 6 mm. and 9 mm. outside diameter. *F* is a connecting tube $1^1/_2$ cm. in diameter and about 21 cm. long. *H* is the receiving flask of 250-cc. capacity containing an antibump. *K* is an antibump constructed from 3-mm. glass rod on the one end of which is sealed a piece of 3-mm. glass tubing 5 mm. long. Its length should be such that it stands vertically in the flask. The inset shows the detail of the entrainment trap which has been designed for this distillation. On leaving the flask, vapors enter *L* and pass up into the large chamber in which partial condensation occurs. Three small holes (*M*)0.2 mm. in diameter are located at the top of the lower entry tube, but not in it, in order to permit the condensed liquid to flow back into the flask. Any microburet of at least five-cc. capacity and readable to fiftieths is satisfactory for the titration.

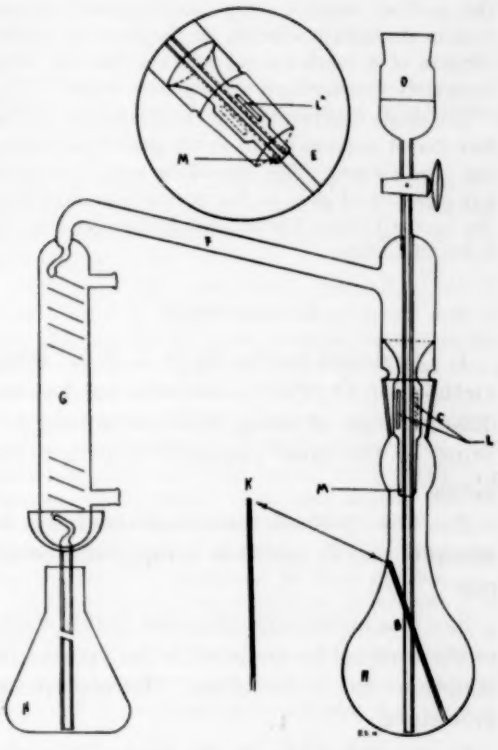

Fig. 2—Drawing showing details of construction of distillation apparatus.

Table I.—Comparative Results of Thyroid Assays

Material	Per Cent Iodine — U. S. P. XI Method	Micro-Method
Thyroid Substance	0.2037	0.2090
	0.2040 Av.	0.2024 Av.
	0.2043	0.1993
C. T. Thyroid 1/3 gr. plain	0.0367	0.0366
	0.0365 Av.	0.0365 Av.
	0.0362	0.0364
C. T. Thyroid 1 gr. S. C. Brown	0.0782	0.0785
	0.0786 Av.	0.0786 Av.
	0.0789	0.0786
C. T. Thyroid 1 gr. Granulation	0.0738	0.0739
	0.0738 Av.	0.0752 Av.
	0.0738	0.0766
C. T. Thyroid 2 grs. Granulation	0.0831	0.0836
	0.0834 Av.	0.0837 Av.
	0.0836	0.0838

Since this method has proved itself to be comparatively simple and to take but a fraction of the time spent on other assays, its accuracy remains as the only point to be confirmed. The Research Surgery Department of Ohio State University found the alkaline ash method to yield consistently high results. Tippett's (2) finding that this was due to a personal equation and could be eliminated by equalizing the titration time of the blank with that of the assay proper made nearly perfect iodine recoveries possible. The authors, to prevent any personal favoring of results, enlisted the aid of their laboratory co-worker, Mr. Roger F. Maize, in checking their work. The results of three operators shown in the following table are in remarkably close accord. Although usually a matter of personal preference, the authors suggest that one-hundredth-normal sodium thiosulfate solution be prepared by careful dilution of a tenth-normal solution that has been accurately standardized against pure copper (3).

The single disadvantage of the method lies in the fact that it requires an all-Pyrex glass apparatus of the type shown. This should be easily made by any experienced glass worker or can be ordered from the Leonard Glass Works, 1432 Minnesota Avenue, Columbus, Ohio.

CONCLUSIONS

1. In skilled hands, the U. S. P. XI assay method for Thyroid is accurate, but has the disadvantage of being time-consuming because of the time required to titrate the blank.

2. The method described is fully as accurate and in addition is rapid and easily run.

3. The authors recommend that a study of the method be made with the view of its adoption as a standard Pharmacopœial procedure.

Credit is due Mr. H. W. Jones, Scientific Director of The Columbus Pharmacal Company, for his invaluable assistance in many phases of this investigation.

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An Interferometer Method for the Assay of Nitrous Oxide

*By Frederick K. Bell and John C. Krantz, Jr.**

For a number of years, the gas interferometer has been used successfully for the analysis of carbon dioxide in air with an accuracy of 0.01 per cent. The fundamental factor which determines the feasibility of the method is the ratio of the indices of refraction of the gases involved and in this case the refractivity[1] of air is 2917×10^{-7} and that of carbon dioxide is 4498×10^{-7}. The value of this ratio also determines the degree of sensitivity that can be expected.

In a survey of the field of possible methods for the assay of nitrous oxide it occurred to the authors that the interferometer method should offer considerable promise. The

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[1] It will be recalled that the refractivity, R, is equal to the index of refraction, n, minus unity, therefore, $R = n - 1$.

The authors are greatly indebted to Carl Zeiss, Inc., of New York, for their generous loan of the interferometer used in these experiments and to the Ohio Chemical and Manufacturing Co. of Cleveland, Ohio, who have supplied us with a considerable number of specially prepared analyzed samples of nitrous oxide.

purity rubrics of the United States Pharmacopœia for this gas eliminate appreciable amounts of all foreign substances with the exception of the gases of the atmosphere and of these only oxygen and especially nitrogen are of importance.

The refractivity of nitrous oxide is 5160 $\times 10^{-7}$, that of air is 2917 $\times 10^{-7}$ and that of nitrogen is 2972 $\times 10^{-7}$. It is thus seen that the conditions for the interferometric analysis of nitrous oxide are even more favorable than for the air-carbon dioxide system. Furthermore, whether the impurity is air or nitrogen or a mixture of these gases, there will be no significant effect, for pharmacopœial purposes, on the accuracy of the analysis.

These predictions have been verified by actual analyses which involved the examination of a large number of analyzed nitrous oxide samples containing from 0.0 to 10 per cent nitrogen and also a number of commercial products. All of these samples have been previously analyzed by one or more of the following methods: (1) water solubility, (2) explosion and (3) condensation. Our results indicate that the interferometric method is accurate to within 0.2 per cent and therefore compares favorably with other methods of assay that have been suggested.

It is the purpose of this communication to describe a simple method for the calibration of the interferometer and to detail a procedure for the assay.

EXPERIMENTAL

The interferometer used was a standard portable Zeiss instrument. The optical system of this compact instrument is so arranged that the optical path traverses the gas chamber twice so that the effective length of the gas cell is twice the actual length. Our first experiments were made using a 50-cm. cell (effective length 100 cm.) which represents the optimum sensitivity for the instrument. It was soon found that in order to take full advantage of this optimum sensitivity considerable refinement and careful regulation of the procedure were required. However, we were able to determine the minimum cell length which could be expected to give the desired sensitivity and a 10-cm. cell (effective length 20 cm.) was selected. The instrument for this size of cell is considerably more compact and less expensive and represents a definite advantage in the ease and rapidity of the procedure.

For the calibration of the interferometer, we have adopted the method of Edwards.[2] A convenient and simple arrangement is shown schematically in Fig. 1. One arm of the glass manometer, *M*, having a length of not less than 50 cm. is open to the atmosphere. The other arm is connected through a glass *T*-tube to the inlet of cell *F* of the gas chamber of the interferometer. The other arm of the *T*-tube is connected to another *T*-tube one arm of which leads to the inlet of cell *E* of the gas chamber. *U*-tubes containing calcium chloride and soda lime, respectively, are connected to this *T*-tube as shown. The outlet tubes of both cells of the gas chamber are joined by a *Y*-tube which leads to a calcium chloride tube. A glass stop-cock is placed at *A* and *B*, *C* and *D* are screw clamps.

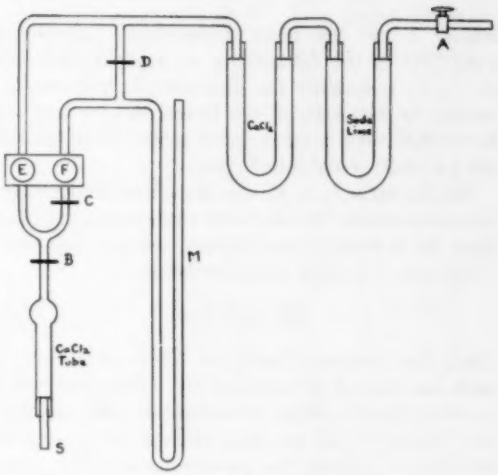


Fig. 1—Apparatus for calibration of interferometer.

The manometer *M* is equipped with a millimeter scale and is filled with any convenient liquid having a low vapor pressure and a known density of approximately unity.

With stop-cock *A* and screw clamps *B*, *C* and *D* open, suction is applied at *S*. A moderate flow of dry, CO_2-free air is drawn through the system for thirty minutes. Clamp *B* is then closed and stop-cock *A* is opened.

At this point the zero reading of the interferometer is determined. Several separate readings should be made and these should check within one division of the drum. The zero reading will be found to lie within a few divisions (plus or minus) of the actual zero of the drum. All interferometer readings are made to the nearest drum division.

To proceed with the calibration, stop-cock *A* is closed and clamp *B* is opened and gentle suction is carefully applied at *S* until the manometer registers approximately 50 cm. pressure difference. Clamps *B*, *C* and *D* are closed and stop-cock *A* is opened.

[2] Edwards, J. D., *J. A. C. S.*, 39, 2382 (1917) and Bureau of Standards, Technologic Paper No. 131 (1919).

The manometer pressure is read to the nearest millimeter and the interferometer reading is made immediately thereafter. By carefully regulating clamp D a new pressure is established in cell F. This new pressure is read on the manometer and the interferometer reading is taken at once. Proceeding in this manner at pressure intervals of from 35 to 40 mm. a series of drum readings of the interferometer and corresponding pressure readings is obtained. The drum readings are corrected for the zero readings and the pressure readings are converted into millimeters of mercury. The room temperature is recorded.

The above data are applied to the equation:

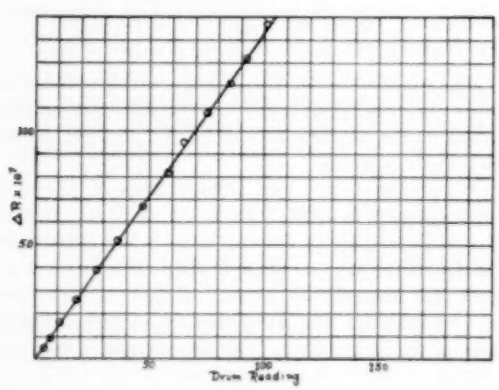
$$\Delta R = \frac{273 \times 0.0002917}{760 \times T} (P_1 - P_2) \qquad (1)$$

where T = the room temperature (absolute); 0.0002917 is the refractivity of dry, CO_2-free air; $P_1 - P_2$ represents the difference in pressure between the two cells of the interferometer and ΔR is the difference in refractivity between the gases in the two cells of the interferometer.

For the term $P_1 - P_2$ we may substitute the manometer pressure Pm (in millimeters of mercury) and then, for a definite temperature, we may introduce a constant C and the equation becomes

$$\Delta R = C\,Pm \qquad (2)$$

Using this relation, a value of ΔR is calculated for each manometer pressure of the calibration series to which there is also a corresponding drum reading. The values of ΔR are then plotted in rectangular coördinates against the corresponding drum readings corrected for the zero reading (Curve I). The points thus obtained should be very nearly on a straight line which passes through the origin and the most probable straight line is drawn through them and the origin.



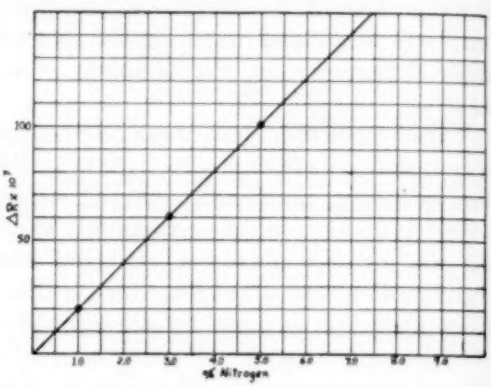
Curve I—Drum readings and refractivity.

A second curve is plotted from values obtained from the following equation:

$$\Delta R = \frac{273}{T} \cdot \frac{P}{760} \cdot \frac{a}{100} (R_1 - R_2) \qquad (3)$$

where T = temperature (absolute)
P = barometric pressure
R_1 = refractivity of nitrous oxide
R_2 = refractivity of nitrogen
a = per cent of nitrogen
ΔR = difference in refractivity between nitrous oxide and nitrous oxide containing a per cent of nitrogen.

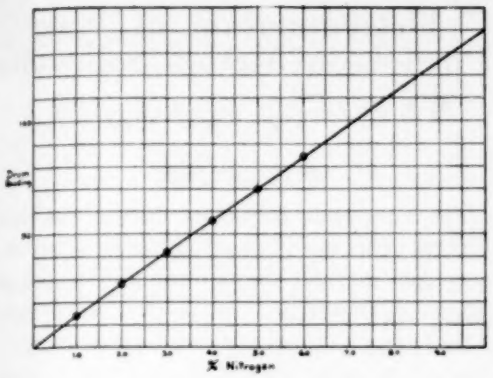
The value of $R_1 = 0.0005160$ and $R_2 = 0.0002972$. Since this equation is also that of a straight line through the origin, a few points will definitely locate its position. Therefore the value of ΔR is calculated for three values of a, *e. g.*, 1, 3 and 5, and these values of ΔR are plotted on rectangular coördinates against the corresponding values of a and the straight line is drawn through the points (Curves II and III).



Curve II—Per cent nitrogen and refractivity.

Curve I and Curve II are now combined so as to yield a third curve in which the drum reading of the interferometer is plotted against the percentage of nitrogen (Curve III) and it is this curve which is to be used in this assay.

The assay is carried out in the following manner. A commercial cylinder of the gas which passes the Tests of Purity of the Pharmacopœia is inverted and, with the valve open wide, liquid nitrous oxide is collected in a suitable container. After approximately one-half of the liquid nitrous oxide has boiled away, the container C is connected to the interferometer system as is shown in Fig. 2. A vigorous flow of nitrous oxide is allowed to pass through the interferometer for several minutes and then an insulating jacket is brought up around the container. Under these conditions there should be a small but steady flow of nitrous oxide through cell E of the interferometer. The exit tube, consisting of 5-mm. glass tubing, proceeds vertically from the container C for approximately 60 cm. and thence horizontally for 20 cm. and finally downward for approximately 90 cm. ending in a mercury seal, S, having a mercury head of 2–3 mm. Approximately 20 cm. above this point a side arm leads to a calcium chloride drying tube and thence to cell E of the interferometer. The exit tube of this cell is connected directly to an anti-diffusion and overflow device as shown. A vertical length of 2.5-mm. glass tubing approximately 70 cm. long is spliced to a short length of 5-mm.



Curve III—Per cent nitrogen and drum reading.

tubing into which is sealed a side arm carrying a mercury seal, the mercury head being 2–3 mm.

The cylinder of gas to be tested, placed in a vertical position and allowed to come to room temperature, is connected to the calcium chloride tube which leads to the inlet tube of cell *F* of the interferometer. The outlet of this cell is connected to an anti-diffusion and overflow device similar to the one just described.

The valve of the cylinder is opened gradually until an over flow is observed at the mercury trap and the gas is allowed to flow at this rate for 15 seconds, after which time the cylinder valve is turned off and clamp *D* is closed. After an interval of 15 seconds the interferometer reading is made and then clamp *D* is opened again. This same procedure is repeated for subsequent determinations either on the same gas sample or on a different sample. The actual time required to completely flush out cell *F* will, of course, vary with the characteristics of the individual set-up and can be readily determined by varying the time until consecutive readings can be checked within 2 drum divisions.

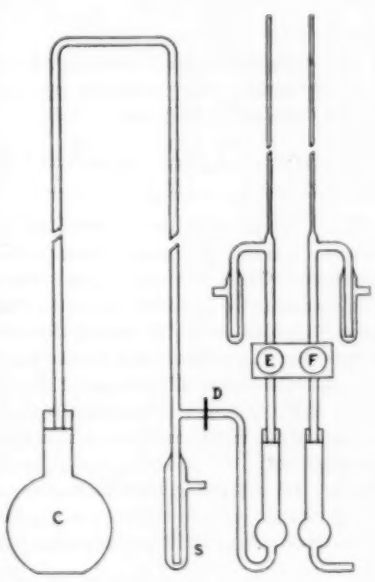


Fig. 2—Schematic interferometer system.

The barometer pressure and the room temperature are recorded. From the drum reading, corrected for the zero reading, of the interferometer, the percentage impurity, as nitrogen, is read off directly to the nearest tenth of a per cent from Curve III.

The results of a series of analyses are shown in Table I. Four determinations were made on each sample before proceeding to the next sample and the determinations are tabulated in the order in which they are made. This series is typical except for the fact that the samples were deliberately selected in a sequence that would show alternately high and low degree of purity. The results indicate that the 15-second period of flushing out the interferometer is adequate and therefore an actual analysis can be performed in approximately one minute.

Table I.—Percentage Impurity as Nitrogen

$t = 22°$; barometer = 764. Zero reading = + 3

Sample	Drum Reading	Drum Reading Corrected	Impurity, %	Purity, %
No. 52, Commercial	19	16	1.1	98.9
	20	17	1.2	98.8
	19	16	1.1	98.9
	19	16	1.1	98.9
No. 50, 9.2 per cent N_2	135	132	9.4	90.6
	135	132	9.4	90.6
	134	131	9.3	90.7
	135	132	9.4	90.6
No. 61, 100 per cent N_2O	5	2	0.2	99.8
	4	1	0.1	99.9
	5	2	0.2	99.8
	4	1	0.1	99.9
No. 95, 8.5 per cent N_2	120	117	8.3	91.7
	119	116	8.3	91.7
	120	117	8.3	91.7
	119	116	8.3	91.7
No. 70, Commercial	17	14	1.0	99.0
	17	14	1.0	99.0
	17	14	1.0	99.0
	17	14	1.0	99.0
No. 27, 4.9 per cent N_2	74	71	5.1	94.9
	74	71	5.1	94.9
	73	70	5.0	95.0
	74	71	5.1	94.9
No. 23, 0.2 per cent N_2	4	1	0.1	99.9
	4	1	0.1	99.9
	5	2	0.2	99.8
	4	1	0.1	99.9
No. 50, 9.2 per cent N_2	134	131	9.3	90.7
	135	132	9.4	90.6
	134	131	9.3	90.7
	135	132	9.4	90.6
No. 61, 100 per cent N_2O	4	1	0.1	99.9
	5	2	0.2	99.8
	4	1	0.1	99.9
	4	1	0.1	99.9
No. 27, 4.9 per cent N_2	73	70	5.0	95.0
	73	70	5.0	95.0
	73	70	5.0	95.0
	74	71	5.1	94.9

The effect of variations in room temperature and barometric pressure on the accuracy of the method

can be readily determined from the equations given above. In Equation 1, it is seen that the barometric pressure does not appear, but a change in 3° in temperature causes a variation of approximately 1 per cent of the value of ΔR. This effect is vanishingly small in the case of purer samples of nitrous oxide and in the case of a 5 per cent nitrogen impurity where the drum reading for the Zeiss instruments is approximately 70, the variation is considerably less than the limit of reproducibility of the drum reading. Two calibrations carried out at different selected temperatures should therefore cover the range of normal seasonal temperature variations.

Equation 3 can be solved for any desired temperature and pressure. The variations in ΔR with temperature are the same as in the case of Equation 1. A variation of 8 mm. in the barometric pressure causes a variation of approximately 1 per cent of the value of ΔR.

The total volume of the interferometer system should be restricted as far as practicable in favor of the efficiency and rapidity with which it can be swept out. The inner walls of all rubber tubing connections should be freed of dust and the exits of the calcium chloride tubes should be well plugged with cotton to filter out all calcium chloride dust.

The actual reading of the interferometer is doubtless a matter of individual variation. We have found, however, that in making readings, eyestrain is greatly reduced if the reading is made as accurately as possible within ten or fifteen seconds. After the eye is rested a few seconds, the final reading is made. Prolonged staring into the instrument is to be avoided. In making several determinations on the same gas sample it is desirable to rotate the drum 10 or 20 divisions in either direction from the previous reading before making a subsequent reading.

The zero reading of the interferometer should be checked from time to time especially when any unusual temperature changes have occurred. Any gas or gas mixture can be used for this purpose, the requirements being that both cells of the interferometer contain the same gas at the same temperature and pressure.

SUMMARY

1. A method for the assay of nitrous oxide based on the use of the gas interferometer has been described.

2. A simple method for the calibration of the interferometer has also been indicated.

3. The interferometric method of assay yields results accurate to within 0.2 per cent and therefore compares favorably with other proposed methods in this respect. The method is unusually rapid.

Determination of Free Phenols in Methyl Salicylate

*By R. W. Towne, R. M. Hitchens and M. S. McCauley**

INTRODUCTION

On numerous occasions during the last several years need has arisen for an accurate, sensitive method for determining traces of phenolic impurities in methyl salicylate. The Dodge (1) method, used extensively, is limited in value since it is sensitive to only 0.02 per cent phenol and since it does not preclude errors caused by volatilization of some salicylic acid along with the phenols. Because of these faults this method is unsatisfactory in cases where it is desired to know the exact phenol content.

EXPERIMENTAL

Principle of Proposed Method.—Since methyl salicylate is itself a phenol, the determination of traces of phenol in this product resolves itself into the separation of two phenols. Fortunately, methyl salicylate is a much weaker acid than phenol itself, although both are extremely weak acids. It should be possible to effect a concentration of the phenol present by extracting the ester with a dilute solution of sodium hydroxide, thus removing all of the phenol together with a little of the methyl salicylate. The reactions:

$$C_6H_5OH + NaOH \rightleftarrows C_6H_5ONa + H_2O$$

$$C_6H_4OHCOOCH_3 + NaOH \rightleftarrows C_6H_4.ONa. + COOCH_3 + H_2O$$

The small amount of methyl salicylate extracted may be converted into sodium salicylate and methanol by saponification:

$$C_6H_4.ONa.COOCH_3 + H_2O \rightarrow C_6H_4.OH.COONa + CH_3OH$$

The excess alkali may be removed by acidification to a p_H value of 9, at which point the phenol will be present largely as such with a small amount of the sodium salt, the salicylate as sodium salicylate and the methanol as such. If the solution is buffered at this p_H value the phenol may be distilled quantitatively and thus separated completely from the salicylate. The phenol in the distillate may be determined by the customary volumetric conversion to tribromphenol.

Details of Proposed Method.—Solutions and Reagents:

(1) Sodium hydroxide solution, 1 Gm. per 100 cc. water.

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(2) Sodium hydroxide solution, 20 Gm. per 100 cc. water.

(3) Hydrochloric acid, concentrated.

(4) Sodium borate, 2 Gm. $Na_2B_4O_7.10H_2O$ per 100 cc. water.

(5) 0.1*N* bromide-bromate solution, standardized against potassium iodate through sodium thiosulfate, 0.1*N*.

(6) 0.1*N* sodium thiosulfate solution, standardized against potassium iodate.

(7) Potassium iodide, AR.

(8) Thymol blue, 0.1 Gm. per 100 cc. made as the one-half monosodium salt in aqueous solution.

(9) Benzene of good quality. If quality is in question wash thoroughly with 1 per cent sodium hydroxide solution before use.

Procedure.—Weigh 25 Gm. of methyl salicylate a 125-cc. pear-shaped separatory funnel. Add 45 cc. benzene. Add 20 cc. of sodium hydroxide solution, 1 Gm. per 100 cc. water. Agitate thoroughly for a few seconds. Allow to separate. Drain off the aqueous layer through a cotton plug in a small, short-stem funnel into a 250-cc. beaker. Make two more extractions with 20-cc. portions of the sodium hydroxide solution. To the combined extracts add 5 cc. of a solution of sodium hydroxide, 20 Gm. per 100 cc. of water. Heat on the steam-bath for 30 minutes. Cool in an ice-bath to 20° C.

Add a few drops of thymol blue indicator. Add a slight excess of hydrochloric acid with constant stirring, keeping the solution in the ice-bath to prevent volatilization of phenol. Redissolve any precipitated salicylic acid with a drop or so of the 1 per cent sodium hydroxide solution, adjusting the acidity so that the solution has a greenish color.

Transfer to a 250-cc. round-bottom flask fitted with a S.T. 19/38 ground glass joint (Fig. 1). Rinse the beaker with 40 cc. of sodium borate solution, 2 Gm. per 100 cc. and add to the flask. Connect the flask to a short spray column filled with small glass helices and fitted with a reservoir at the top of the column. Distil into a 250-cc. glass-stoppered flask. When the volume of the solution being distilled has been reduced to 30 cc. add 20 cc. of water and redistil to a 30-cc. volume.

To the distillate add 10.00 cc. of 0.1*N* bromide-bromate solution. This is sufficient to take care of 0.05 per cent phenol. If the phenol content is in excess of 0.05 per cent, proportionately more bromide-bromate must be added. If more than 20 cc. is required, repeat the analysis with a sample sufficiently small to bring the titration below 20 cc. Add 4 cc. concentrated hydrochloric acid. Let stand five minutes at not over 25° C. Add 1 Gm. potassium iodide. Rinse the stopper carefully. Titrate with 0.1*N* sodium thiosulfate solution to a soluble starch end-point.

$$\text{Per cent Phenol} = \frac{(\text{cc. } 0.1N \text{ bromate} - \text{cc. } 0.1 \text{ thio}) \times 100 \times 0.001567}{\text{weight of sample}}$$

Notes: The possibility of decomposition of sodium salicylate to phenol and sodium carbonate during the analysis was studied by distilling a solution of borax and sodium salicylate. The complete absence of phenolic bodies in the distillate is evidence that decomposition does not take place. That methyl alcohol does not interfere was proved by running a blank with a large excess of methanol.

Verification of the Proposed Method.—Phenol-free methyl salicylate for the preparation of knowns was made by washing methyl salicylate repeatedly with 1 per cent sodium hydroxide solution. A total of six such washings was made even though the third washing was found to be phenol-free.

Fig. 1—Phenol distillation apparatus.

The efficiency of the alkali extraction during analysis was determined by adding a known amount of phenol to the phenol-free material and then determining the phenol content of each extract. It was found, with 0.0138 per cent phenol added, that about 90 per cent was removed by the first extraction and the remainder by the second. None whatever could be detected in the third. When the three extracts were combined for distillation 0.0139 per cent phenol was recovered.

Similar knowns were made by adding various amounts of phenol to the phenol-free product and were analyzed by the suggested procedure. The results are given in Table I.

Table I.—Determination of Phenol in Known Mixtures of Phenol and Methyl Salicylate

Test No.	Per Cent Phenol Added	Per Cent Phenol Found	Error, Per Cent Phenol
1	0.0037	0.0040	+0.0003
2	0.0073	0.0075	+0.0002
3	0.0138	0.0139	+0.0001
4	0.0220	0.0218	−0.0002
5	0.0220	0.0216	−0.0004
6	0.0440	0.0439	−0.0001

CONCLUSION

The method described for the determination of traces of phenolic impurities in methyl salicylate is both rapid and accurate. It permits the detection of as little as 0.001 per cent free phenolic bodies and is reproducible to ±0.0003 per cent. This is a decided improvement over the customary method which is sensitive only to 0.02 per cent phenol.

REFERENCE

(1) Dodge, F. D., *Drug Markets*, 22 (1928), 609.

A Method for the Determination of Calomel in Tablets

By R. A. Bosee and L. A. Perlenfein†*

The well-known reaction which occurs when calomel is heated in the dry state with sodium carbonate or bicarbonate may be used to assay mercurous chloride in tablets. The method is especially useful for those tablets in which the presence of other ingredients renders the iodine method inapplicable, and makes filtration of the entire tablet mass difficult.

$$Hg_2Cl_2 + 2NaHCO_3 \rightarrow 2Hg\uparrow + 2NaCl + CO_2\uparrow + H_2O\uparrow$$

The method was first used in our laboratories for the assay of calomel and soda tablets.

EXPERIMENTAL

Assay for Calomel.—Weigh not less than 20 of the tablets, reduce them to a fine powder without an appreciable loss, transfer an aliquot portion equivalent to about 0.5 Gm. of mild mercurous chloride to a nickel crucible and ignite. Leach the carbonized mass with boiling water, add 30 cc. of tenth-normal silver nitrate, 5 cc. of nitric acid and filter. To the filtrate add ferric ammonium sulfate T.S. and titrate the excess of silver nitrate with tenth-normal ammonium thiocyanate.

Each cc. of tenth-normal silver nitrate is equivalent to 0.02361 Gm. of mild mercurous chloride, HgCl.

Assay for Sodium Bicarbonate.—It is also possible to obtain a figure for both sodium bicarbonate and calomel on the same sample. After igniting and leaching with boiling water, the filtrate can be first titrated with normal sulfuric acid in the presence of methyl orange, and then a chloride titration run.

The number of cc. of normal sulfuric acid multiplied by 0.08401 represents the amount of sodium bicarbonate not entering into the reaction with the calomel. The figure obtained for calomel multiplied by 0.356 represents the quantity of sodium bicarbonate entering into reacton. The sum of these two results gives the entire amount of sodium bicarbonate present in the sample.

If the method is to be used for assaying calomel in tablets not containing soda, carbonate or bicarbonate may be added and either titration used. If the alkalimetrical titration is used, a definitely measured quantity of reagent must be added. If the chloride titration is used, an excess of the reagent is all that is necessary. In either case, however, care must be exercised that the calomel does not volatilize before it has a chance to react with the carbonate. Best results were obtained by adding a few drops of water and mixing into a smooth, thick paste. In the case of the calomel and soda tablets the thorough mixing of the ingredients and the compression of the tablets make the addition of moisture before igniting unnecessary.

Procedures similar to both of those given have been used before for the determination of calomel in aqueous media. M. Kohn (1), (2), (3) showed that mercuric halides could be decomposed in alkaline solution and the halogens subsequently determined by the method of Volhard. D. Köszegi (4) pointed out that calomel could be determined by treating with a known quantity of normal NaOH, filtering and titrating the remaining NaOH with normal acid. However, for the usual calomel tablets, heating in the dry state with carbonate furnishes a quick and accurate method of eliminating troublesome fillers and at the same time volatilizes the metallic mercury formed during the reaction.

Results.—The following are examples of the results obtained by following these procedures:

Results of Assay of Calomel Tablets

Weight of Sample, Gm.	Gm. Calomel Calculated[a]	Found	Error, %
1.5056	0.4926	0.4925	0.02
1.5022	0.4914	0.4880	0.72
1.5040	0.4920	0.4904	0.32
Weight of Sample, Gm.	**Gm. Sodium Bicarbonate Calculated[a]**	**Found**	**Error, %**
1.5056	1.0017	0.9940	0.77
1.5022	0.9997	0.9966	0.31
1.5040	1.0009	0.9996	0.13

[a] Calculated amounts are based on assay of each separate ingredient by U. S. P. method.

REFERENCES

(1) Kohn, M., *Zeit. anorg. Chemie*, 59 (1908), 108–110.

(2) Kohn, *Ibid.*, 59 (1908), 271.

(3) Kohn, M., and Ostersetzer, A., *Ibid.*, 80 (1913), 218–220.

(4) Köszegi, D., *Pharm. Zeit.*, 76 (1931), 524.

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Assay of Sulfanilamide in Tablets

*By W. E. Honsinger and R. E. Schoetzow**

In the control testing of uncoated sulfanilamide tablets, made with the usual excipients, it is necessary that the sulfanilamide content be determined, and for that purpose it is desirable that a method be used which results in the isolation of the sulfanilamide so that its purity and identity can be demonstrated by means of the melting point. This also serves to confirm the correctness of the assay, at least that it is not too high since, if so, a low melting point would result.

Difficulty was encountered in the isolation of sulfanilamide from tablets by the use of solvents. Sulfanilamide is only very slightly soluble in ether, chloroform or ethylene dichloride, and it is difficultly soluble in petroleum ether and benzene. It is soluble in acids and bases, but cannot be separated from alkaline or acid solution by ether or chloroform. It is more soluble in ethyl alcohol and methyl alcohol. Attempts to take advantage of this fact by using a mixture of alcohol and chloroform to extract the sulfanilamide from a watery suspension of sulfanilamide and the tablet excipients failed to yield pure sulfanilamide. Extraction of the dry powder with alcohol alone likewise resulted in assay residues of low melting point. When these residues were washed with an ice-cold, saturated aqueous solution of sulfanilamide, residues of the proper melting point were obtained, but the residues were lower than theory in weight. It was finally found that if the dry mixture of sulfanilamide and tablet excipients was first freed of soluble material other than sulfanilamide by extraction with a cold, saturated aqueous solution of sulfanilamide and then the sulfanilamide recovered from the residue by extraction with hot alcohol an assay residue could be recovered from the alcohol solution which is close to the proper weight and melting point.

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EXPERIMENTAL

The method finally adopted and used with success in the quantitative determination of sulfanilamide in tablets is as follows:

Accurately weigh 20 tablets on counterpoised watch-glasses with an analytical balance, transfer them to a clean mortar, and powder. Accurately weigh 0.4 Gm. of the powder with an analytical balance and transfer it to a 25-cc. glass-stoppered graduated cylinder. Add 10 cc. of an ice-cold, filtered, saturated solution of sulfanilamide. Shake well. Filter through a Jena glass filter (fritted glass filter). Wash any residue in the cylinder and the material on the filter with a few drops of ice-cold distilled water to displace any sulfanilamide solution. Completely extract the residue in the cylinder and the material on the filter with 80 to 100 cc. of hot alcohol. Wash the funnel tip and any exposed edges with a few cc. of hot alcohol. Collect the alcohol and washings in a tared, 250-cc. beaker. Carefully evaporate the alcohol on a steam-bath. Dry in the oven at 80° C. to constant weight. The weight of the residue so obtained must correspond to not less than 92 and not more than 108 per cent of theory (5-gr. tablet).

Determine the melting point of the assay residue by the U. S. P. XI method. This must be not less than 163° C. and not more than 167° C.

The following table gives the results of assays and the melting points of the assay residues on ten samples of 5-gr. tablets:

Melting Point of Assay Residue

Assay, Per Cent of Theory	Melting Point of Assay Residue
95.47	163.3–164.4° C.
93.09	164.3–164.8° C.
98.37	164.0–164.7° C.
93.09	163.5–164.6° C.
98.77	163.4° C.
96.92	164.9–165.9° C.
92.33	165.0–165.7° C.
101.24	164.4–164.9° C.
99.06	163.0–164.0° C.
93.29	164.5–165.5° C.

SUMMARY

A method for the assay of sulfanilamide in uncoated tablets has been presented, in which the purity of the isolated sulfanilamide is shown by its melting point. We suggest that in the development of assays of medicinal substances the assay should, if possible, be such that it will result in the isolation of the active principle and the purity of which can be determined by a melting-point determination or other suitable test.

Fluorescence Analysis as Applied to Some Alkaloids and Crude Drugs*

By A. Slesser† and C. B. Jordan‡

PART I—ALKALOIDS

In the course of routine analysis it is sometimes advantageous to apply rapid tests for identity or purity of materials. For example, a rapid method of identification of the components present in a mixture of alkaloids would be quite desirable.

Analysis by fluorescence methods has, during the past few years, been successfully applied for rapid qualitative and quantitative analysis of chemicals and drugs. The method is comparatively simple; the only apparatus required is a source of ultraviolet rays and a screen to filter out the visible rays so that any fluorescence shown by the substance under examination can be easily discerned when the experiment is performed in a dark room.

Where the fluorescence produced is characteristic of the substance irradiated, it may be used as a means of analysis. Ultraviolet rays are desirable for fluorescence analysis because (1) they stimulate fluorescence in many substances, and (2) they are, in themselves, invisible and do not mask any fluorescence produced.

The source of the ultraviolet rays used in the work was a quartz mercury-vapor arc and a control unit so devised that a constant operating voltage, and thus a constant output of ultraviolet rays, could be maintained. A light-proof hood to encase the arc was an essential part of the apparatus. To an aperture in the bottom of the hood was fitted a special glass filter to remove the visible, and yet allow the passage of ultraviolet rays, chiefly those having a wavelength of 3650 Å.

Table Showing Results of Fluorescence Analysis

				Precipitate with						
				A	B	C	D	E	F	G
Number	Alkaloid	Sulfate	Hydrochloride	Picrolonic Acid	Mayer's Reagent	Wagner's Reagent	Picric Acid	Phosphotungstic Acid	Phosphomolybdic Acid	Tannic Acid
1	Quinine	Blue	Weak	Neg.	Neg.	Neg.	Neg.	—	Neg.	Neg.
2	Strychnine	Weak	Weak	Neg.	Neg.	Neg.	Neg.	—	Neg.	Neg
3	Brucine	Weak	Weak	Neg.	Neg.	Neg.	Neg.	—	Neg.	Neg.
4	Hydrastine	—	—	GY	Neg.	—	Neg.	—	Neg.	—
5	Hydrastinine	—	WB	Neg.	Neg.	—	Neg.	IW	Neg.	—
6	Emetine	—	Blue	Neg.	Neg.	—	Neg.	—	—	—

WB = whitish blue, GY = golden yellow, IW = intense white.

EXPERIMENTAL

Initial experimental attempts were devoted to examination of the fluorescence of several alkaloids before and after purification. The following alkaloids were studied: quinine, strychnine, hydrastine, hydrastinine and emetine. The purpose of this part of the work was (1) to note if differences either in color or intensity of fluorescence were shown after recrystallization, and (2) to prepare comparatively pure forms of the alkaloids for subsequent salt formation.

The results indicated that three recrystallizations did not alter to any great extent the color or intensity of the fluorescence. The presence of moisture in the sample, however, did lessen the intensity, as evidenced by the increase in intensity after drying.

The second portion of the experimental work was performed with the object of ascertaining whether or not corresponding salts of similar alkaloids showed the same type of fluorescence; if the fluorescence differed, two similar alkaloids, *e. g.*, strychnine and brucine, could be detected in the presence of each other by adding the proper reagent and examining under the lamp. The salts formed were those yielded by a solution of the alkaloid upon treatment with the common alkaloidal precipitating reagents: Wagner's Reagent, Mayer's Reagent, picric acid

* An abstract of a thesis presented to Purdue University in partial fulfilment of the requirements for the Master of Science degree by A. Slesser, 1937.

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(saturated aqueous solution), tannic acid (10% aqueous solution), phosphotungstic acid, phosphomolybdic acid and picrolonic acid (1% alcoholic solution).

The results of examination under the lamp are shown in the preceding table.

The alkaloidal precipitants themselves showed no fluorescence in ultraviolet light.

A dash in the table indicates either that no precipitate was formed or else the amount formed was too slight to handle.

Each of the precipitates was next examined in three different acids, namely, acetic, sulfuric and hydrochloric.

SUMMARY AND CONCLUSIONS

(1) The nature of fluorescence of several alkaloids before and after purification has been noted.

(2) The presence of moisture in the powder was found in certain cases to influence the color and intensity of the fluorescence.

(3) Precipitates of the alkaloids with common alkaloidal precipitating reagents have been examined under quartz light and were found with a few exceptions to show no visible fluorescence, thus making impossible identification or differentiation by this method.

(4) The appearance in ultraviolet light of the alkaloidal precipitates in different solvents has been carried out.

PART II—CRUDE DRUGS

Nearly all crude drug extracts display luminescence in ultraviolet light, the intensity of color usually increasing on dilution of the solution up to a certain point. The color depends on the fluorescently active substances present in the extract, provided, of course, that the solvent is itself non-fluorescent. Capillary analysis applied to the extracts will show a number of colored zones on the filter paper, but the color visible to the eye under the ultraviolet rays may be due to only one substance, which exhibits a more intense fluorescence than any of the others present.

Alcoholic extracts of the drug ipecac on dilution show an intense bluish white luminescence in reflected, and a greenish color in transmitted ultraviolet light. There is no record in the literature that the causative agent for the luminescence has been sought. It was of scientific interest to the writer to attempt to isolate and, if possible, identify the fluorescently active principle.

EXPERIMENTAL

An alcoholic extract of coarsely powdered Brazilian ipecac was prepared by the percolation method, the alcohol removed by heating on a steam-bath, extraneous matter removed by the usual method of precipitation with lead acetate solution and the lead removed with hydrogen sulfide gas. The resulting filtrate displayed the characteristic colors under the lamp. A portion of the liquid was treated with Mayer's Reagent and the precipitate filtered off. The resulting filtrate displayed no luminescence. This seemed to indicate that alkaloidal material was responsible for the fluorescence.

The alkaloids occurring in ipecac are emetine, cephalline, psychotrine, *o*-methyl psychotrine and emetamine. Of these the first two are present in much more abundant quantities than any of the others.

The removal of emetine and cephalline from the other alkaloids, which was accomplished by making use of the ready solubility of these two alkaloids in ether and the insolubility of the others in this solvent, did not alter in any way the color or intensity of fluorescence of the filtrate. This seemed to be a pretty good indication that the luminescence was not due to emetine or cephalline.

The deëmetinized filtrate was evaporated to low volume under diminished pressure at a temperature not exceeding 50° C. Tests for the presence of glycoside, tannin and flavones performed on the solution were negative. The test for alkaloid, however, was positive.

The evaporation was continued as before, small amounts of alcohol being added from time to time in order to remove all traces of water, and anhydrous ether, to remove the alcohol.

The dark, viscid residue was then extracted successively with the following solvents: petrolatum benzin, anhydrous ether and chloroform. Before each extraction the preceding solvent was removed by evaporation. Of these extracts, only the chloroformic fraction showed the characteristic blue-green color under the lamp.

After several attempts at recrystallization, two crystalline hydrochlorides were obtained from the residue of the chloroform fraction. The microscope showed that prismatic and needle-shaped crystals were present. The latter were removed by solution in absolute alcohol.

The prismatic crystals gave a distinct test for alkaloids, while the needle-shaped ones did not. Neither salt showed fluorescence under the lamp in the dry state, but both gave the intense bluish white color on the addition of a few drops of water.

SUMMARY AND CONCLUSIONS

(1) The substance or substances causing the characteristic fluorescence of alcoholic extracts of ipecac in ultraviolet light is alkaloidal in nature as proved by the disappearance of the fluorescence after alkaloidal precipitation has been effected.

(2) The alkaloid responsible is not emetine or cephalline, because removal of these from the extract in no way alters the color or intensity of fluorescence.

(3) The substance responsible for the fluorescence is not a decomposition product produced by heating at steam-bath temperature because non-heat treated extracts show the same color as the others.

(4) The alkaloid may be psychotrine, *o*-methyl psychotrine or emetamine. Of these, the first two are the more likely suspects because emetamine and solutions of its salts are non-fluorescent in daylight; this argues against the likelihood of their showing such an intense fluorescence as displayed by the extracts of ipecac under quartz light.

(5) Solutions of the unknown substance darken on standing, and deposit a brown substance. Psychotrine solutions are said to act in this manner.

(6) Ether extracts of ipecac do not display the same type of fluorescence that alcoholic extracts show. Psychotrine is insoluble in ether and soluble in alcohol.

(7) Chloroform will extract the fluorescently active material from ipecac. Psychotrine is soluble in chloroform.

(8) Psychotrine or *o*-methyl psychotrine or both are apparently responsible for the fluorescence of the alcoholic extracts of ipecac.

Incompatibilities in Prescriptions

IV. The Use of Inert Powders in Capsules to Prevent Liquefaction Due to Deliquescence*,†

By William J. Husa‡ and Charles H. Becker

In a previous paper (1) a study was made of the effectiveness of various inert powders in capsules in which the contents liquefy due to formation of a eutectic mixture. In the present paper a report is made on the relative efficiency of various inert powders in preventing liquefaction of the contents of capsules due to deliquescence.

EXPERIMENTAL

Prescription No. 1

℞ Salol	℈ ii
Sodii Nitrite	grs. xx
Sodii Bromid	℈ iv
M. ft. caps. no. xxiv.	
Sig.: Take one after meals.	

On the above prescription as obtained from the file of a pharmacy, there appeared a notation indicating that the dispenser had added 40 grs. of lactose.

Upon mixing the ingredients of this prescription as written, a dry powder was obtained and single doses were put in No. 2 capsules. After four days the capsules in a beaker liquefied and acquired a rusty-red color. After a week, the capsules were entirely colored and adhered to the bottom of the container. On longer standing, the capsules hardened, and upon observing the interior, the ingredients were found dry and white in color except for a thin colored layer where the powder was in contact with the capsule. The capsules stored in a tightly stoppered jar had undergone no changes after three weeks. Similar results were obtained when 40 grs. of lactose were added for 24 capsules. The results show that lactose did not prevent liquefaction of the capsules when placed in an open container, whereas, in a tightly stoppered container, lactose was not necessary since the capsules had undergone no change.

The prescription was also filled using various amounts of inert powders. In all cases of compounding, the sodium bromide and sodium nitrite were first triturated together to obtain a fine powder. The inert powder and salol were then added in the order named. The temperature during the time of compounding was between 72° and 73° F. When using from 1 to 5 grs. of each absorbent per individual dose, the size of the capsule ranged from No. 2 to No. 00.

The results on capsules stored in open containers were as follows:

Light Magnesium Oxide and Magnesium Carbonate.—When using as much as 5 grs. of the absorbents per individual dose, the capsules developed a rusty-red color and became soggy and sticky after standing two weeks; the contents were dry.

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* Read before the Section on Practical Pharmacy and Dispensing, A. PH. A., Atlanta meeting, 1939.

† This paper is based on part of a thesis presented to the Graduate Council of the University of Florida by Charles H. Becker, in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy.

‡ Head Professor of Pharmacy, University of Florida.

Lactose.—When using as much as 5 grs. of the absorbent per capsule, liquefaction occurred. All capsules developed a rusty-red color and became soft and sticky after standing several days.

Talc and Heavy Magnesium Oxide.—When using 1 gr. of the absorbents per individual dose, the capsules developed a rusty-red color and became soggy and sticky after standing two weeks; the contents were damp. The use of 2, 3, 4 and 5 grs. of the absorbents per individual dose gave similar results as to the condition of the capsule, but the contents were dry.

Starches Dried at 100° C., Corn Starch, Wheat Starch and Potato Starch.—When using 1 gr. of the absorbents per individual dose, the contents liquefied. The capsules developed a rusty-red color and became soggy and sticky. Using 2, 3, 4 and 5 grs. of the absorbents per individual dose, similar results were observed but the contents were damp.

The results on capsules stored in open containers indicate that none of the inert powders used prevented discoloration and disintegration of the capsules. When stored in closed vials, all capsules containing from one to five grs. of the inert powders remained stable over a period of two weeks. The results indicate that in this prescription it is important to dispense the capsules in tightly closed vials and when this is done it is unnecessary to add an inert powder.

Prescription No. 2

℞ Sodium Nitrite
Sodium Iodide aa gr. iiss
Misce et fiat capsules no. xx.
Sig.: One t. i. d. 1st week then b. i. d. as directed.

This prescription (2), (3), (4) contains two deliquescent drugs. Sodium iodide alone in capsules turned dark, absorbed water and dissolved the capsules within several days. Capsules of sodium iodide in closed containers remained stable over a period of two weeks. Sodium nitrite alone in capsules gave no sign of deterioration after standing two weeks. On compounding the prescription as written and putting individual doses in No. 3 capsules, the contents turned yellow and liquefied after standing two days. Capsules placed in closed containers developed a light yellow color after standing two weeks; the contents, however, were dry.

The prescription as written was also filled using various amounts of inert powders. In all cases of compounding, the sodium iodide was triturated first with the absorbent and then the sodium nitrite was added. The temperature during compounding was between 72° and 73° F. When using from 1 to 5 grs. of each absorbent per individual dose, the size of the capsule ranged from No. 3 to No. 00.

The results on capsules stored in open containers were as follows:

Light and Heavy Magnesium Oxide.—Capsules containing from 1 to 5 grs. of the absorbents per individual dose turned yellow after several days of standing. When using 1 gr. of the absorbents per capsule, the contents liquefied after standing a few days. When using 2 grs. of the absorbents per capsule, the contents developed into a damp mass, but liquefaction did not take place even after standing two weeks; the gelatin capsule itself swelled and became soft and sticky. The contents of the capsule containing 3, 4 and 5 grs. of the absorbents per individual dose remained dry over a period of two weeks, but the gelatin capsule itself became soft and sticky.

Magnesium Carbonate.—Capsules containing from 1 to 5 grs. of magnesium carbonate per individual dose turned yellow after several days of standing. Although the absorbent prevented liquefaction, even when using 1 gr. of the inert powder per capsule, the gelatin capsule itself swelled and became soft and sticky; the contents were dry over a period of two weeks.

Lactose.—As much as 5 grs. of lactose per capsule did not prevent liquefaction. All the capsules turned yellow and, likewise, became soft and sticky.

Talc.—Capsules containing from 1 to 5 grs. of talc per individual dose turned yellow after several days of standing. As much as 3 grs. of the absorbent per capsule did not prevent liquefaction. Five grains of the absorbent per capsule gave a damp mass when standing over a period of two weeks and the gelatin capsule itself swelled and became soft and sticky.

Air-Dried Starches and Starches Dried at 100° C., Corn Starch, Wheat Starch and Potato Starch.—Capsules containing from 1 to 5 grs. of the absorbents per individual dose turned blue after a few days. After standing two weeks, the contents of the capsules developed a rusty-red color. When 1 and 2 grs. of the absorbents per capsule were used, the contents liquefied after standing a few days. When using 3, 4 and 5 grs. of the absorbents per capsule, the contents became damp and the gelatin capsule itself swelled and became damp and sticky after standing two weeks.

The results on capsules stored in closed vials were as follows:

Magnesium Carbonate and Lactose.—Capsules containing up to 5 grs. of the absorbents per individual dose turned yellow after standing a few days. The contents were dry over a period of two weeks.

Talc, Light and Heavy Magnesium Oxides, Air-Dried Starches and Starches Dried at 100° C., Corn Starch, Wheat Starch and Potato Starch.—Capsules containing 1 gr. of the absorbents per individual dose remained stable over a period of two weeks.

None of the inert powders prevented discoloration and deterioration of capsules stored in open containers. However, the prescription can be filled satisfactorily by adding one gr. of starch or magnesium oxide per capsule and dispensing in a closed capsule vial.

Prescription No. 3

℞ Strych. Sulph. gr. 1/40
Ferr. et Ammon. Citrate gr. vii
Ft. caps. Tal. ds. xx.
Sig.: One capsule t. i. d. p. c.

On the above prescription as obtained from the file of a pharmacy, there appeared a notation to the effect that the dispenser had added five grs. of heavy magnesium oxide per capsule, presumably because iron and ammonium citrate is deliquescent in moist air.

Using iron and ammonium citrate from an old stock and filling the prescription as written, the chemicals in the mortar gummed up. It was also noticed that this sample had an ammoniacal odor. When using a fresh stock of iron and ammonium citrate and triturating it with the strychnine sulfate, a fine powder was obtained which was easily put in No. 2 capsules. After four days of standing, the ingredients of the capsules in the open container absorbed water and developed into a soft mass. Upon further standing, the mass shrunk, and after standing two weeks, the mass was dark brown in color, half of the original size and hard as cement.

The capsules in the closed container had undergone no changes after three weeks.

When filling the prescription with 5 grs. of heavy magnesium oxide per capsule, a No. 00 capsule had to be used. Although this made a bulky capsule there was no change in either type of container after standing three weeks. The prescription was also filled using 2 grs. of heavy magnesium oxide per capsule and using a No. 1 capsule. This made a capsule which was stable in both types of containers over a period of three weeks.

In conclusion, heavy magnesium oxide was found to be necessary in preventing the formation of a soft mass when the capsules were placed in an open container, although the inert powder was not necessary to stabilize the capsules when storing them in a closed container.

The prescription was also filled using various amounts of inert powders. In all cases of compounding, the iron and ammonium citrate and the strychnine sulfate were triturated first to obtain a fine powder and then the absorbent was added. The temperature during compounding was between 72° and 73° F. A No. 1 capsule was used for individual doses containing 1 gr. of magnesium carbonate, light and heavy magnesium oxides and talc. When using from 1 to 5 grs. of lactose and the various dried starches per individual dose, the size of the capsule was either No. 1 or No. 0.

For capsules stored in open containers, the results were as follows:

Magnesium Carbonate, Talc and Light and Heavy Magnesium Oxides.—When using 1 gr. of the absorbents per individual dose, the capsules remained stable over a period of two weeks.

Lactose and Starches Dried at 100° C., Corn Starch, Wheat Starch and Potato Starch.—All capsules containing from 1 to 5 grs. of the absorbents per individual dose developed into a mass of cement-like hardness after two weeks of standing. The contents shrunk considerably in all capsules.

When stored in closed vials, all capsules containing from 1 to 5 grs. of the inert powders remained stable over a period of two weeks.

Prescription No. 4

℞ Methenamine
Potassium Acetate
Prepare thirty capsules, containing two grs. of each component.

When filled as written, this prescription (5) yields a damp powder which liquefies in capsules within a few days. Due to removal of moisture from the gelatin capsules by the deliquescent potassium acetate, the capsules become brittle and crack within a few minutes after filling. The contents of capsules stored in closed vials were dry over a period of two weeks; however the capsules become brittle and cracked.

The prescription as written was filled using various amounts of inert powders. In all cases of compounding, the methenamine was first triturated to obtain a fine powder and then the absorbent and potassium acetate were added in the order named. The temperature during compounding was between 72° and 73° F. When using from 1 to 5 grs. of each absorbent per individual dose, the size of the capsule ranged from No. 1 to No. 00.

The results on capsules stored in open containers were as follows:

Magnesium Carbonate and Light Magnesium Oxide.—When using 1 and 2 grs. of the absorbents per individual dose, the capsules became brittle within a few minutes after compounding and cracked. After standing a few days, the contents liquefied, and the gelatin capsule itself became soggy and sticky. The contents of capsules containing 3, 4 and 5 grs. of the absorbents per individual dose remained dry over a period of two weeks, but the gelatin capsule itself became sticky.

Heavy Magnesium Oxide.—When using 1 and 2 grs. of the absorbent per individual dose, the capsules became brittle within a few minutes after compounding and cracked. After standing a few days the contents liquefied, and the gelatin capsule itself became soft and sticky. Using 3 grs. of the absorbent per capsule, similar results were observed, except that the contents were slightly damp after standing two weeks. The contents of capsules containing 4 and 5 grs. of the absorbent per individual dose remained dry over a period of two weeks, but the gelatin capsule itself became soft and sticky.

Lactose.—As much as 5 grs. of the absorbent per individual dose did not prevent liquefaction and the gelatin capsule itself became soft and sticky.

Talc, Air-Dried Starches and Starches Dried at 100° C., Corn Starch, Wheat Starch and Potato Starch.—When using from 1 to 4 grs. of the absorbents per individual dose, the capsules became brittle

within a few minutes after compounding and cracked. After standing a few days the contents resulted in a pasty mass, and the gelatin capsule itself became soggy and sticky. Five grains of the absorbents per capsule gave similar results, except that the contents were damp after standing two weeks.

When the capsules were kept in closed vials, the following results were obtained:

Lactose.—When using from 1 to 3 grs. of the absorbent per capsule, the contents became damp and developed into a mass of cement-like hardness after standing two weeks; the gelatin capsule itself became brittle and cracked. Capsules containing 4 and 5 grs. of the absorbent per individual dose remained stable over a period of two weeks.

Talc, Light and Heavy Magnesium Oxides, Magnesium Carbonate, Air-Dried Starches and Starches Dried at 100° C., Corn Starch, Wheat Starch and Potato Starch.—All capsules containing from 1 to 5 grs. of absorbent per capsule remained stable over a period of two weeks, with the exception that, when smaller amounts of the absorbents were used, some of the capsules were brittle and cracked.

To stabilize prescription No. 4 it is thus necessary to use an inert powder and to dispense the capsules in a tightly closed capsule vial.

Prescription No. 5

℞ Acetophenetidin	ʒ ss
Fldext. Gelsemium	♏ iii
Citrated Caffeine	gr. xii
Sodium Bromide	ʒ ss

Mix. Make 12 capsules.

On compounding this prescription as written, the capsules in an open container remained stable for several days but on longer standing the contents became slightly damp and the capsule itself became soft and sticky. Capsules placed in a closed vial remained stable over a period of two weeks. Capsules of sodium bromide alone gave similar results.

The prescription was also filled using various amounts of inert powders. In all cases of compounding, the sodium bromide was triturated first to obtain a fine powder and then the absorbent, fluidextract of gelsemium, citrated caffeine and acetophenetidin were added in the order named. The temperature during compounding was between 70° and 71° F. When using 1 gr. of magnesium carbonate and light magnesium oxide per individual dose, a No. 1 capsule was used. When using from 1 to 5 grs. of talc, lactose, heavy magnesium oxide and the various dried starches per individual dose, the size of the capsule was either No. 1 or No. 0.

The results on capsules stored in open containers were as follows:

Light Magnesium Oxide and Magnesium Carbonate.—Capsules containing 1 gr. of the absorbents per individual dose were stable over a period of two weeks.

Lactose.—Although lactose prevented liquefaction even when using 1 gr. of the absorbent per individual dose, as much as 5 grs. per capsule did not prevent the contents from developing into a pasty mass after standing two weeks; also, the gelatin capsule itself became very soggy and sticky.

Talc, Heavy Magnesium Oxide and Starches Dried at 100° C., Corn Starch, Wheat Starch and Potato Starch.—When using 1 gr. of the absorbents per individual dose, the contents remained dry over a period of two weeks, but the gelatin capsule itself became soggy and sticky. Even when using 5 grs. of the absorbents per capsule, similar results were obtained.

All capsules remained stable over a period of two weeks when kept in a closed vial.

To stabilize prescription No. 5, it is necessary to add one gr. per capsule of magnesium carbonate or light magnesium oxide but such addition is unnecessary if the capsules are dispensed in a tightly closed vial.

Prescription No. 6

℞ Sodium Bromide	gr. xl
Antipyrine	gr. xxvi
Camphor	gr. iv
Citrated Caffeine	gr. vi
Tinct. Aconite	gtt. xii

Mix and make 12 capsules.

Sig.: One capsule every three or four hours.

This prescription contains two ingredients, antipyrine and citrated caffeine, which result in a sticky mass on standing a few hours and also contains a deliquescent chemical, sodium bromide. On compounding the prescription as written, a damp powder was obtained and individual doses were put in No. 1 capsules. After standing four days exposed to the air, the gelatin capsule itself became sticky and the contents, likewise, were of a pasty nature. Capsules placed in closed containers remained stable over a period of two weeks.

The prescription was filled using various amounts of inert powders. In compounding the prescription, the camphor and tincture of aconite were triturated until most of the alcohol was volatilized and then the inert powder was added. The antipyrine, sodium bromide and citrated caffeine were added, having first been triturated to a fine powder, and the final mixture was mixed lightly. The temperature at the time of compounding was between 78° and 80° F.

Table I shows that talc, lactose and the various dried starches were inefficient as inert powders. The interior of the capsules containing various amounts of these inert powders was of a pasty consistence after standing two weeks, and the gelatin capsule itself was rubber-like in texture and sticky. The contents of capsules containing 2 and 3 grs. of heavy magnesium oxide per capsule, developed into a mass of cement-like hardness and the gelatin capsule itself was sticky. The contents of capsules containing 1 and 2 grs. of light magnesium oxide and magnesium carbonate were dry, but the gelatin capsule itself became sticky after standing two weeks; when 3 grs. of these inert powders per cap-

Table I.—Prescription No. 6 with Inert Powders
Capsules Stored in Open Containers

Abbreviations: L. = Liquid of Soft Mass; P. = Dry Powder; D. = Damp Powder; S. D. = Slightly Damp Powder; C. M. = Cement-Like Mass.

Inert Powder, Grs.	Size of Capsule	Time in Days 0	1	2	4	14
Heavy Magnesium Oxide						
1	1	S. D.	S. D.	S. D.	L.	D.
2	1	P.	C. M.	C. M.	C. M.	C. M.
3	0	P.	P.	C. M.	C. M.	C. M.
Light Magnesium Oxide						
1	1	S. D.	S. D.	S. D.	L.	D.
2	0	P.	P.	P.	P.	P.
3	0	P.	P.	P.	P.	P.
Magnesium Carbonate						
1	1	S. D.	S. D.	S. D.	L.	L.
2	0	P.	P.	P.	P.	P.
3	0	P.	P.	P.	P.	P.
Talc						
1	1	S. D.	D.	D.	L.	L.
2	1	S. D.	D.	D.	L.	L.
3	0	S. D.	S. D.	S. D.	L.	L.
Lactose						
1	1	S. D.	D.	D.	L.	L.
2	1	S. D.	S. D.	D.	L.	L.
3	0	S. D.	S. D.	S. D.	L.	L.
Dried Corn Starch						
1	1	S. D.	D.	D.	L.	L.
2	1	S. D.	D.	D	L.	L.
3	0	S. D.	S. D.	S. D.	L.	L.
Dried Wheat Starch						
1	1	S. D.	D.	D.	L.	L.
2	1	S. D.	S. D.	D.	L.	L.
3	0	S. D.	S. D.	S. D.	L.	L.
Dried Potato Starch						
1	1	S. D.	D.	D.	L.	L.
2	1	S. D.	D.	D.	L.	L.
3	0	S. D.	S. D.	S. D.	L.	L.

sule were used, the capsules remained in a stable condition over a period of two weeks.

Capsules stored in closed vials were still in good condition after two weeks.

A question arose as to whether traces of powder adhering to the outside of capsules had any effect in hastening liquefaction. To obtain data on this point prescription No. 6 was filled as written and a little of the powder was sprinkled on the outside of some of the capsules while others were cleaned thoroughly to remove any particles that might have adhered to them. In open containers, capsules having powder on the outside absorbed water and became sticky within a day; after two days the contents were damp and the capsules themselves were sticky and soft. Capsules having no adhering powder were stable for three days; after four days the contents were pasty and the capsule was sticky. These results indicate that, in case of capsules containing deliquescent substances, the presence of powder adhering on the outside of the capsule hastens deterioration.

DISCUSSION OF RESULTS

The present study was devoted to capsules containing deliquescent drugs. The cause of liquefaction in such cases is primarily the absorption of moisture by the deliquescent substance. If some of the ingredients are soluble in water, liquefaction is hastened. Some deliquescent drugs are capable of absorbing so much moisture from the gelatin capsule that the capsules become brittle and crack. Usually, however, the chief factor is the absorption of moisture from the air; the fact that an airtight container stabilized almost all of the capsules verifies this.

Capsules containing deliquescent drugs should be dispensed in glass capsule vials and, if this is done, it is usually unnecessary to add an inert powder. In most instances, none of the inert powders used were effective in stabilizing capsules placed in open containers. However, the addition of an inert powder sometimes makes the capsules more stable. As to the relative efficiency of the various powders, magnesium carbonate and light magnesium oxide were best, heavy magnesium oxide and talc ranked next, and lactose and the starches were poorest. Drying of the starches at 100° C. did not seem to be of any practical advantage.

It is of interest to note that magnesium carbonate and light magnesium oxide, which were the most efficient inert powders for capsules containing deliquescent drugs, were also the most efficient powders in capsules in which the contents liquefied due to formation of a eutectic mixture (1).

The use of glass capsule vials is imperative in dispensing capsules containing deliquescent substances but, if liquefaction is due to formation of a eutectic mixture, the type of container is of no importance as far as stability of the capsule is concerned (1).

SUMMARY

A study was made of the effects of the use of inert powders in capsules in which liquefaction occurs due to deliquescence of the ingredients. In some cases the addition of an inert powder increased the stability of the capsules to a certain extent; best results were obtained with magnesium carbonate and light magnesium oxide, next best results were secured with heavy magnesium oxide and talc, and poorest results were given by lactose and starch.

In most instances the use of inert powders did not stabilize capsules stored in open containers. However, in most cases it was found unnecessary to add any inert powder in prescriptions for capsules containing deliquescent drugs, provided that the capsules were dispensed in airtight glass containers. Hence it is imperative to use an airtight container, such as a screw-top glass capsule vial, in dispensing capsules containing deliquescent substances.

In case of capsules containing deliquescent substances, the presence of powder adhering on the outside of the capsule hastens deterioration.

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A Micro-Colorimetric Method for the Determination of Copper in Ampules of Iron, Arsenic and Copper

By Roland A. Bosee and Paul Fehder†*

INTRODUCTION

In the course of control analysis of ampule products containing organic combinations of iron, arsenic and copper, used in the treatment of hypochromic anemias, a reliable method for separating and estimating micro-quantities of copper was devised and corroborated. The original procedure worked out by a former associate[1] was based on a micro-colorimetric method (1) for determining traces of copper in blood and milk. This procedure was modified by a simple preliminary treatment to separate the copper-ion from the organic compounds of iron and arsenic. The copper was determined by the development of the cupric sulfocyanate-pyridine complex, soluble in chloroform and possessing a characteristic green color.

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EXPERIMENTAL

Procedure.—Measure an exact aliquot of the sample solution equivalent to approximately 0.40 or 0.45 mg. of copper, into a small beaker, add 0.5 cc. of concentrated hydrochloric acid and dilute with distilled water to a volume of about 25 cc. Saturate the solution with hydrogen sulfide at room temperature until the mixture turns milky and a deposit of copper sulfide and sulfur is formed. Filter through a small retentive filter paper (C. S. & S. No. 595). Wash the residue with several portions of hot distilled water. Reject the filtrate and washings which contain the iron and arsenic.

Dissolve the copper sulfide by pouring several portions of boiling 25% nitric acid into the filter. Collect the filtrate (containing cupric nitrate) in a 100-cc. beaker. Wash the filter several times with hot distilled water, collecting the washings in the beaker containing the filtrate. Evaporate the filtrate, at first on a hot plate to a small volume, and finally on a steam-bath. Keep the beaker covered with a ridged watch glass during the evaporation to avoid contamination with dust or cinders. To the residue of cupric nitrate, add 5 cc. of concentrated hydrochloric acid and again evaporate to dryness on a steam-bath.

Dissolve the cupric chloride residue in several drops of $N/1$ hydrochloric acid. A clear solution should be formed. Add 2 cc. of distilled water and transfer the mixture to a 25-cc. glass-stoppered volumetric flask. Rinse the beaker with four 2-cc. portions of distilled water and transfer the rinsings to the flask. The total volume of solution should not exceed 10 cc.

Prepare a copper standard solution by measuring exactly 5 cc. of a Standard Copper Solution, containing 0.1 mg. Cu in each 1 cc. of solution, into a second 25-cc. glass-stoppered volumetric flask. Add to this flask the same number of drops of $N/1$ hydrochloric acid as added to the cupric chloride residue. Dilute with distilled water to the same volume (not more than 10 cc.). The Standard Copper Solution is prepared by dissolving exactly 392.8 mg. of clear, *uneffloresced* crystals of reagent grade cupric sulfate, $CuSO_4.5H_2O$, in exactly 1000 ml. of distilled water.

Now add 2 drops of phenolphthalein T.S. to both flasks and titrate each with $N/1$ sodium hydroxide to a pink end-point. Following this, add to each flask, in succession, exactly 10 cc. of chloroform, 1 cc. of glacial acetic acid, 1 cc. of 10% potassium sulfocyanate solution and 15 drops of pyridine, medicinal grade. Dilute both flasks to the mark with distilled water, stopper and agitate thoroughly. When the chloroform fractions containing the dissolved copper

sulfocyanate-pyridine complex separate, transfer each mixture to dry separatory funnels. Permit the chloroformic and aqueous layers to separate completely. Drain about 4 cc. of each of the chloroformic fractions into individual colorimetric comparison cups of a Duboscq colorimeter. Compare the green colors as to relative intensity. Take an average of ten readings rapidly.

$$\frac{\text{Reading of standard (mm.)} \times 0.5 \text{ mg. of Cu}}{\text{Reading of sample (mm.)} \times \text{Volume of sample taken for assay}} = \text{mg. Cu per 1 ml. sample}$$

Discussion.—The method presented above has given consistently accurate results with average deviations of plus 3.7% and minus 2.0% from the theoretical quantities of copper assumed to be present. Aside from experimental and manufacturing errors, the possibility of some exsiccation in the copper salts used in the manufacture of these ampule products may be a contributing factor to such deviations. The following tables are compiled from the assay reports of the different lots of ampules prepared during the year 1939, and are presented to prove the accuracy of the method:

Table I.—Copper Content of Ampule No. 175-A[a]

Lot No.	Copper Content, in mg., per 10 Cc. Ampule
10,875	0.314
10,986	0.309
11,200	0.313
11,260	0.313
11,446	0.307
11,536	0.291
11,666	0.284
11,822	0.294
11,987	0.296
12,038	0.308
12,129	0.298
12,197	0.307
12,345	0.298
Average	0.3025

Table II.—Copper Content of Ampule No. 175[a]

Lot No.	Copper Content, in mg., per 5 Cc. Ampule
10,897	0.147
10,925	0.158
11,003	0.166
11,283	0.155
11,504	0.152
11,599	0.146
11,764	0.145
11,805	0.155
11,985	0.161
12,030	0.154
12,233	0.145
Average	0.153

[a] The theoretical quantity of copper is 0.300 mg. Cu per 10 cc. Ampule Solution.

Table III.—Copper Content of Ampule No. 345[a]

Lot No.	Copper Content, in mg., per 2 Cc. Ampule
11,263	0.420
11,388	0.418
11,558	0.417
11,986	0.410
Average	0.416

Table IV.—Copper Content of Ampule No. 345-B[a]

Lot No.	Copper Content in mg., per 1 Cc. Ampule
10,882	0.198
11,218	0.210
11,583	0.202
Average	0.203

[a] The theoretical quantity of copper is 0.200 mg. Cu per 1 cc. Ampule Solution.

It has been observed in some instances that the shade of green color shown by the copper sulfocyanate-pyridine complex produced by the sample is a trifle more yellow than that shown by the standard copper solution complex. Traces of iron or sulfur alter the shade of green color. However, the intensities can be definitely compared without difficulties. Several attempts to obtain comparable readings by treating the standard copper solution aliquot by the same procedure used in separating and extracting the copper in the ampule sample produced inconsistent and low results.

SUMMARY

1. A suitable method for estimating micro-quantities of copper in ampules of iron, arsenic and copper has been devised, based on a micro-colorimetric method employed in biochemistry for estimating traces of copper in blood and milk (1).

2. The results of thirty-one determinations indicate that the method is sufficiently accurate and reliable to warrant its use in estimating micro-quantities of copper salts in medicinal preparations.

REFERENCE

(1) Hawk and Bergeim, "Practical Physiological Chemistry," 10th Edition (1931), page 468.

Book Reviews

Biological Products, by LOUIS GERSHENFELD, P.D., B.Sc., Ph.M. Publishers, Romaine Pierson, New York. 236 pages, price $4.00.

The author is professor of Bacteriology and Hygiene at the Philadelphia College of Pharmacy and Science. He has prepared the work with a purpose to give information on the preparation, manufacture, uses and other essentials of bacteriology and their practical application; to serve students as a guide for their studies, and for those who carry on laboratory work. It grew out of the need of the author in his field and gives particularly useful information on antitoxins, serums, vaccines for those in the allied public health professions. The book is illus-

trated with plates of a number of pharmaceutical manufacturing establishments to whom the author expresses appreciation. Thirty chapters comprise the Table of Contents. The book is well bound and contains many bibliographic references in the text. A criticism is offered on the typography; parts of the book in the reviewer's opinion, would have been improved in appearance if the type were clearer and some pages would be read with greater ease and assurance. There seems to have been an effort to hold down the number of pages by the use of small type. Bold face small type makes the reading of important matter somewhat difficult for those who are not very familiar with the subject.—E. G. EBERLE.

The Essentials of Physiology and Pharmacodynamics, by GEORGE BACHMANN, M.S., M.D., Professor of Physiology in the School of Medicine of Emory University; sometime Demonstrator of Physiology in the Jefferson Medical College, Philadelphia, and A. RICHARD BLISS, Jr., Ph.D., M.D., LL.D., Professor of Pharmacology and Dean of Pharmacy in Howard College of Birmingham, Ala., member of the Committee of Revision of the U. S. Pharmacopœia. Third Edition. Publishers, The Blakiston Co., Philadelphia. Price, $4.50. Published, January 2, 1940. The edition has been thoroughly revised.

Use has been granted in this volume by the respective Revision Committees of U. S. Pharmacopœia and National Formulary, also the British Pharmacopœia, and New and Nonofficial Remedies, for comment—no responsibility is accepted by the respective committees, for inaccuracies or errors in percentage strengths. The authors have placed the titles of individual drugs and preparations at the end of the groups; the aim is to present the material for study by students of pharmacy, including the essential facts of anatomy. The principles of pharmacodynamics are explained and the relation of materia medica with notes on pharmacology, hygiene and public health. The book contains 506 pages, 196 illustrations. Typography and binding are good. The revisers state that they have considered especially those topics in which more important advances have been made, as the vaccines, electro cardiogram, the pharmacodynamics of the cell, the vitamins, the barbiturates, newer antiseptics, newer anesthetics, the endocrines, allergens. The Table of Contents shows 42 chapters; the authors are known to teachers of pharmacy, they have prepared the revision for use in pharmacy schools, and libraries of pharmacy and other professional institutions, and for individuals.—E. G. EBERLE.

A Textbook of Materia Medica, Pharmacology and Therapeutics, by HAROLD N. WRIGHT, M.S., Ph.D., Assistant in Pharmacology, University of Minnesota; and MILDRED MONTAG, R.N., M.A., Instructor in Nursing Arts, St. Luke's Hospital, New York City, illustrated. Published by W. B. Saunders Co., Philadelphia and London, 560 pages. Price $2.75.

The authors state that the subject matter for the study of drugs is commonly divided into Materia Medica, Pharmacology, Therapeutics and Toxicology. They have drawn upon their teaching experience for drugs used in the presentation of the text, and for the explanation of the use of weights and measures in the calculation of doses and in making preparations. The book gives information on toxicology, antiseptics, oxygen therapy, new preparations, endocrines, vitamins and on sulfanilamide and sulfapyradine. The standards under the Food and Drugs Act are briefly described. The biographies and illustrations are well selected. For some description, articles could have been selected from the PROCEEDINGS OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. More National Formulary preparations could have been used. Under Narcotics, marihuana could have been employed for illustrating the possibilities of addiction and the responsibilities of those who handle narcotics. Marihuana is not used in prescription practice. The authors, wherever they had opportunity, give brief and clear illustrations. This applies to solutions and other forms of Materia Medica wherein calculations are required, prescription dosage and parts when additions or reductions are to be made on order of the physician. The authors have prepared a useful work, they have different views on some questions, as an example, the use of the designation of "dram" is a subject of discussion at different times. "Dram" is not frequently used and is not essential to medical and pharmaceutical practice, but possibly more should have been said in connection with the subject.—E. G. EBERLE.

The Second Supplement to the Pharmacopœia of the United States of America, Eleventh Decennial Revision (U. S. P. XI—1939 Supplement.) *1939*. Distributed by the Mack Printing Company, Easton, Pennsylvania, 178 pages. Price, $1.50.

The Second Supplement to U. S. P. XI became official on January 1, 1940, and all monographs and general tests published in this Second Supplement superseded the corresponding U. S. P. XI Standards and First Supplement on that date with exceptions for the revised minimum standards for vitamin A under *Oleum Morrhuæ* and under *Oleum Morhuæ Non-Destearinatum*, and also for the new monograph for Chorda Chirurgicalis—these will become official July 1, 1940. The new articles under English title are: Ascorbic Acid, Mandelic Acid, Nicotinic Acid, Tribasic Calcium Phosphate, Surgical Gut, Cyclopropane, Tribasic Magnesium Phosphate, Methylrosaniline Chloride, Soluble Pentobarbital, Sulfanilamide, Thiamine Hydrochloride, Natural Vitamin A in Oil, Natural Vitamin A and D in Oil. Changes in official Latin titles are: Ergosterol Activatum in Oleo, Petrolatum Liquidum, Petrolatum Liquidum Leve, Serum Antipneumococcicum. Changes in official English titles are: Antipneumococcic Serum, Liquid Petrolatum, Light Liquid Petrolatum, Activated Ergosterol in Oil. All page

references in this Supplement refer to U. S. P. XI unless otherwise indicated. Glass-stoppered bottles may differ from those specified if resistant to the substance packaged, apparatus is subject to related specification. Specifications for the content of the bottle for Solution of Magnesium Citrate are given. Arsphenamine and Neoarsphenamine labels must bear expiration dates—Arsphenamine to be not more than five years from date of manufacture, that of Neoarsphenamine not more than three years.—E. G. EBERLE.

Medicolegal and Industrial Toxicology, Criminal Investigations, Occupational Diseases, by HENRY J. EILMANN, Ph.D., Director of Physicians' Laboratory Service of Toledo, Ohio, Lecturer in Bacteriology and Histology, Mary Manse College of Toledo. Publishers, The Blakiston Company, Philadelphia. Price $3.00. Published January 3, 1940.

The drugs designated poisons have been classified according to chemical behavior, during isolation from mixtures. Four groups are presented; the first includes volatile substances and acetone, analine, chloroform, hydrocyanic acid, phosphorus, etc. The members of the second group are separated with alcohol containing tartaric acid, *e. g.*, acetanilid, picric acid, salicylic acid; another sub-group, by ether extraction after the acid has been rendered alkaline, includes alkaloids. Still another sub-group obtained by separating alkaloids, in the ether-chloroform solution and rendering alkaline with amonium hydroxide includes apomorphine, morphine, narceine, etc. The third group contains arsenic, antimony, bismuth, mercury, lead, silver, etc. In group four are mineral acids, alkalies and organic drugs. The general plan is a study of the symptoms of poisoning, the toxicology, detection, quantitative determination and postmortem findings. Other studies include criminal investigations, medicolegal examinations, industrial poisoning and the last, occupational diseases—classification of diseases under the "Compensation Act." The work has been prepared for those who seek information on the subjects presented, and is of value to industrial physicians, lawyers, insurance adjusters, toxicologists and laboratory workers, for general information and those who seek information on all phases of court cases, not only poisonings, but also other means of accidental or unlawful injury or destruction.—E. G. EBERLE.

Accepted Foods, The Council on Foods of the A. M. A. xx + 492 pages, 5 1/2 x 8 1/2. American Medical Association, Chicago, Illinois. Price $2.00.

This volume comprises a list of foods accepted by the American Medical Association Committee on Foods, particularly the trade marked foods of the present-day diet. It contains such articles as oleomargarine with fish liver oil, cookies of thiamine hydrochloride, beverages of ascorbic acid, milk with ergosterol, etc. It will no doubt do in time for foods what "New and Non-official Remedies" has done for trade marked medicinals.—A. G. D.

German-English Science Dictionary, by LOUIS DEVRIES. x + 473 pages, 5 x 7. McGraw-Hill, New York, 1939. Price $3.00.

The author is Professor of Languages at Iowa State College and it is stated that the dictionary is intended for use in the agricultural, biological and physical sciences. The volume contains forty-eight thousand German scientific terms and will be found to be sufficiently comprehensive for all practical purposes. There is an appendix which contains definitions for some of the more troublesome German abbreviations.—A. G. D.

Merck's Index, fifth edition, by JOSEPH ROSIN, C. V. ADDINALL, ALFRED I. CONE and other members of the Merck staff. 1060 pages, 6 x 9, Merck & Co., Rahway, New Jersey, 1940. Price $3.00.

The first edition of this encyclopedia for drugs and chemicals was published in 1889 and succeeding editions in 1896, 1907, 1930 and 1940. The volume contains descriptions of nearly six thousand drugs and chemicals and includes a discussion of such topics as cold-tar colors for use in food, drugs and cosmetics, culture media, fixatives, staining solutions, etc. It also contains tables of physical constants, indicators and other information of a similar nature. The entire series of "Tests and Reagents by Author's Names" published in *Merck's Report* has been included in the appendix. The list comprises 4500 tests. There is also a table of antidotes. The monographs as heretofore are short and concise and include for the first time structural formulas for organic substances. The book is a useful source of information to anyone dealing with drugs and medicinal chemicals and particularly the pharmacists.—A. G. D.

May's Chemistry of Synthetic Drugs, by PERCY MAY and G. MALCOLM DYSON, fourth edition. xii + 370 pages, 5 3/8 x 8 1/2. Longmans, Green & Co., New York. Price $6.00.

The fourth edition of this book makes its appearance seventeen years after the publication of the third edition. The authors begin with a discussion of the theories of the action of drugs, the effects of various organic radicals and the changes which drugs undergo in the organism. They then discuss in systematic order the anesthetics, narcotics, antipyretics, alkaloids, the tropeines, the isoquinolines, ethylamines, hormones, vitamins, phenols, halogens, organometallic compounds and others. In each instance, brief descriptions are given as to the structure, properties and uses of the compounds. Specific methods of preparation of compounds are not given although in most instances general methods of preparation are stated. The book is an excellent one for obtaining a general view of the present state of development of the chemistry of synthetic medicinal substances.—A. G. D.